U.S. DAIRY FORAGE RESEARCH CENTER

1990 RESEARCH SUMMARIES

U.S. Dairy Forage Research Center
1925 Linden Drive West
Madison, Wisconsin 53706

Agricultural Research Service

United States Department of Agriculture

March 1991

U.S. DAIRY FORAGE RESEARCH CENTER, USDA-ARS
Madison, WI 53706

Dear Reader:

It is a pleasure to update our progress by bringing you these summaries of recent research. The U.S. Dairy Forage Research Center is a unique part of the national research program of the Agricultural Research Service, U.S. Department of Agriculture. The Center's mission is to build a knowledge and technology base for the dairy industry to fully exploit the use of forages in the production of milk. The Center has agricultural engineers, plant and soil scientists, microbiologists, ruminant nutritionists and a chemist working together to increase the efficiency of forage production and utilization by dairy farmers. We function in close cooperation with the Agricultural Experiment Stations of several states. The Center is located on the campus of the University of Wisconsin, Madison, and has "Cluster" locations in St. Paul, MN, Ames, IA, East Lansing, MI, and Ithaca, NY. The Center's research farm with facilities for 300 milking cows is located on 63 acres of USDA land on the banks of the Wisconsin River in Prairie du Sac, WI. An additional 1200 acres of adjacent land is utilized by the Center by agreement with the U.S. Department of the Army. The Center was established in 1980 and has made steady growth since. At present there are fifteen scientists: ten at Madison, and one at each of three Cluster locations, and two at the St. Paul, Minnesota Cluster location. Scientists hold faculty appointments in university departments and provide supervision for approximately 20-25 graduate students and 4-8 post doctoral fellows.

The Agricultural Research Service (ARS) has been struggling to provide adequate support to its scientists in recent years because annual appropriations have not kept up with inflationary increases in costs. As a consequence, there has been a significant reduction in the number of programs supported by ARS. We are sad to report that three of our cluster programs were amongst the reductions. The programs in Pennsylvania, Ohio and Missouri were discontinued this year, and consequently Drs. Abrams, Shockey and Martz and their colleagues have had to seek positions outside of ARS. At this writing, all have secured other positions. We thank them for the contributions they have made and for the time with us. We wish them well in their new positions. We were able to obtain significant increases in budget for the remaining cluster scientists through this overall program of downsizing, so net effect on the U.S. Dairy Forage Research Center budget was small.

We are awaiting the arrival of the Nuclear Magnetic Resonance (NMR) instrumentation any day now. This was a very major capital investment (about $400,000) that we feel will greatly enhance the Center's program in the area of forage and cell wall chemistry. The cell wall work group (Buxton, Hatfield, Jung, Mertens, Ralph and Weimer) is laying a very good foundation for significant progress in our understanding of the chemical architecture of the cell wall. Hopefully this can provide leads for genetic manipulation of forages to increase the availability of cell wall constituents to the ruminant animal. The International Symposium on The Forage Cell Wall, planned for October, 1991, has stimulated much interest, and we are looking forward to hosting approximately 150-200 international scientists in Madison next fall.

We are pleased and very proud of the way Center scientists from diverse disciplines interact and bring their collective insights to bear on the problems of forage production and utilization. This collection of research summaries illustrates the progress they are making in developing information to help dairy farmers utilize their resources more effectively. The research is intended to benefit producers of forage crops, dairy farmers and the consumers of dairy products.

Sincerely,

Larry D. Satter

Larry D. Satter, Director
U.S. Dairy Forage Research Center

U.S. DAIRY FORAGE RESEARCH CENTER STAFF

G.A. Broderick*
Research Dairy Scientist
(608)264-5356
FTS: 364-5356

D.R. Buxton
Research Plant Physiologist
USDA/ARS/USDFRC
Department of Agronomy
Iowa State University
Ames, IA 50011
(515) 294-9654
FTS: 865-9654
FAX: (515)294-3163

R.D. Hatfield*
Research Plant Physiologist
(608)264-5358
FTS: 364-5358

H.G. Jung
Research Dairy Scientist
USDA/ARS/USDFRC
Department of Agronomy and
Plant Genetics
411 Borlaug Hall
University of Minnesota
St. Paul, MN 55108
(612)625-8291
FAX: (612)625-1268

R.G. Koegel*
Research Agricultural Engineer
(608)264-5149
FTS: 364-5149

D.R. Mertens*
Research Dairy Scientist
(608)264-5228
FTS: 364-5228

R.E. Muck*
Research Agricultural Engineer
(608)263-3742
FTS: 364-5245

C.A. Rotz
Research Agicultural Engineer
USDA/ARS/USDFRC
Rm 206, Agric. Engr. Dept.
Michigan State University
East Lansing, MI 48824
(517)353-1758
FTS: 374-6706
FAX: (517)353-8982

J.B. Russell
Research Microbiologist
Wing Hall
Section of Microbiology
Cornell University
Ithaca, NY 14853
(607)255-4508
FAX: (607)255-3904

M.P. Russelle
Soil Scientist
USDA/ARS/USDFRC
Department of Soil Science
1529 Gortner Avenue
University of Minnesota
St. Paul, MN 55108
(612)625-8145
FAX: (612)370-2485

L.D. Satter*
Director and Research Dairy
Scientist
(608)263-2030
FTS: 364-5240

R.R. Smith*
Research Plant Geneticist
(608)262-9752
FTS: 364-5279

L.L. Strozinski
Herd Manager
USDA/ARS/USDFRC
Route #1
Prairie du Sac, WI 53578
(608)643-2438
FTS: 364-5138

J. Ralph*
Research Chemist
(608)264-5407
FTS: 364-5407**R.P. Walgenbach**
Farm Manager
USDA/ARS/USDFRC
Prairie du Sac, WI 53578
(608)643-2438
FTS: 364-5138

P.J. Weimer*
Research Microbiologist
(608)264-5408
FTS: 364-5408

*Madison location address is:
USDA/ARS/USDFRC
1925 Linden Drive West
University of Wisconsin
Madison, WI 53706
(608)263-2030
FTS: 364-5240
FAX: (608)264-5275

Disclaimer

Trade names and the names of commercial companies are used in this report solely to provide specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over products not mentioned.

Acknowledgment

Appreciation is expressed to Gloria Palmer for her interest in and dedication to the task of typing and assembling this annual research summary.

CONTENTS

FORAGE HARVEST AND STORAGE

FORAGE PRESERVATION

RUMEN MICROBIOLOGY

FORAGE UTILIZATION BY CATTLE AND SHEEP

RESEARCH PROCEDURES

FARM/HERD REPORT - WISCONSIN

PUBLICATIONS

DAFOSYM: THE DAIRY FORAGE SYSTEM MODEL

C.A. ROTZ and P.W. WILKENS

Introduction

In 1978, a project was initiated to develop a comprehensive model of the dairy forage system. This model soon became known as DAFOSYM. Development of this model has continued with additions and refinements made each year. The original intent was to create a research tool that could be used to explore and evaluate new technology for forage harvest and storage on the dairy farm. DAFOSYM has served this purpose well. Many systems with both new and old technologies have been evaluated and compared on representative dairy farms.

As DAFOSYM has become a more versatile model, interest has grown in having a version of the model designed for more general use as a decision aid for farmers and farm consultants. A major objective in this year's work with DAFOSYM was to distribute copies of DAFOSYM and obtain information from the users on the usefulness of the model as a decision aid. Work has also continued on the development and refinement of portions of the model.

Materials and Methods

Several new functions or models were added to DAFOSYM during the past year to increase its versatility or usefulness. Major changes included:

1. Addition of a direct cut harvest model for alfalfa silage with formic acid applied to enhance fermentation,
2. Functions for the machine parameters, drying rate and losses from tedding were added to allow simulation of this process,
3. A function was added to modify alfalfa yield as affected by cutting management and persistence,
4. The roughage constraint in the animal model was modified. The new constraint requires that at least 60% of the NDF of the diet come from large particles (essentially forage stem material),
5. A more detailed model of feeding systems was added to determine machinery, fuel and labor requirements in feeding.

New output options were added to DAFOSYM to provide more information to the user. One option provides a more detailed description of the characteristics of each available feed and the total diet of each animal group fed. A new output display also was added which gives the actual cost of producing and feeding each of the major feeds.

Last year (1989) a version of DAFOSYM was completed for general distribution. A reference manual and a user guide were also completed to provide proper documentation of the model. Several articles were published in journals and conference proceedings. These articles brought visibility to the model.

During this year (1990) copies of DAFOSYM, the reference manual and user guide were distributed to those requesting copies. Over 60 copies were distributed. Most went to locations within the U.S. with seven copies going to Canada, two to Italy and one each to South Africa and Australia. For those distributed in the U.S., most were sent to areas in the North Central and Northeast regions, but copies were also sent to Nebraska, Texas, Idaho, Kentucky, California, Oklahoma, Arkansas, Louisiana and North Carolina. These users represented a wide range of backgrounds from research to farming. A breakdown of the users by their primary interest is: Research (33%),

Extension (25%), Teaching (15%), Government (8%), Industry (8%), Consultant (6%), and Farmer (5%). In October a survey was sent to all users to determine how the model was used and the level of interest in further development of DAFOSYM as a decision aid.

Results and Discussion

Survey responses were received from about half of the users. The respondents represented each of the interest groups of the users in approximately the same proportion designated above. Most of the respondents said they had tried the model and 17% said they had actually used the model to evaluate a problem.

Overall, those responding to the survey supported further development of DAFOSYM as a teaching/decision aid tool. Of the respondents, 70% felt DAFOSYM was a significant tool in its current form, 17% felt major revision of the input and output structures were required and 13% were undecided. Eighty-three percent strongly supported further development of DAFOSYM as a decision aid with 17% undecided. Ninety-six percent were interested in receiving new versions of DAFOSYM.

As a result of the information received, DAFOSYM appears to be an effective tool for classroom teaching and extension workshops. It may also have limited usefulness for consultants specializing in dairy farm management, and a few farmers may use the model. Further refinement will improve the usefulness of the model. The primary need is for better methods of editing input data files and displaying output data. Greater ease in obtaining weather information for a given location would also increase its versatility.

Development of a new version of DAFOSYM was initiated to meet these needs. The new model will include:

1. The latest dairy forage simulation model with overlaying input menus,
2. A user friendly editor for modifying input data files,
3. A plotting routine for graphical display of major output options,
4. A weather model for creating typical weather patterns for any location.

This new model should be ready for distribution during the next year (1991).

FORAGE ALFALFA MODEL

P.W. WILKENS, C.A. ROTZ and J.T. RITCHIE

Introduction

Research and modelling efforts were directed at defining and incorporating functional relationships of alfalfa growth and development into the dynamic alfalfa simulation model, FORAGE ALFALFA. Characteristic of this and similar models is a Priestly-Taylor water balance function with a minimum data set that specifies required input variables. Soil profile characteristics and climatic data can be interchanged for collaborative model development and validation experiments. The objective of the model development is linkage into the Dairy Forage System Model (DAFOSYM) to better evaluate alternative crop management and feeding strategies within the framework of a dairy farm.

Materials and Methods

A functional alfalfa model was developed in FORTRAN based on concepts from both CERES-Maize and ALSIM 1 Level 2. Model inputs parallel those of similar legume and grass models developed

within the IBSNAT project. Weather data required includes solar radiation (MJ/m^2), maximum and minimum temperature (°C), and daily precipitation (mm). The soil-water balance model developed by Ritchie requires the lower limit (LL), drained upper limit (DUL), and saturated water content (SAT) for each soil layer . Runoff curve numbers, developed by the Soil Conservation Service, are based on soil textural data and slope. Soil color estimates albedo. A root hospitality factor for each layer is needed for modelling root development. In addition, pH, NO_3, and NH^4 concentrations are designated by soil layer. Organic carbon and plant residue are also required inputs for a nitrogen balance. Genetic parameters required at this time include winter hardiness (degree of cold tolerance), ineffective or effective nodulation, and disease resistance to several prominent pathogens. A complete soil-plant nitrogen balance routine, including dinitrogen fixation, was incorporated as a module in the model. Support programs were developed to allow the easy creation, editing, and plotting of both data input and output files.

Results and Discussion

The major components of the model include vegetative and reproductive growth, water balance, carbon balance, and nitrogen balance procedures. Phasic development, growth, carbon partitioning, and forage quality are a function of regrowth period, temperature, and photoperiod. Functional relationships derived from supporting field experiments are being incorporated into seedling year growth functions. Verification, calibration, and validation of the model follow model and/or module development. Results of one validation experiment are shown in Table 1. Two years of alfalfa yield, quality, and phenological development were compared to simulated values. Biomass production and phenological stage at harvest were generally predicted with good ability. First harvest yield was generally slightly under predicted and last harvest slightly over predicted. Forage fiber and protein fractions were predicted with prediction errors (RMSE) of less than 3 (data not shown). Other sections of the model were validated in independent data sets. Data collected in cooperation with the NifTAL project in Hawaii is being used to calibrate and validate nitrogen fixation. Predicted root growth is being tested in cooperation with Dr. Bob Loomis, University of California, Davis.

Conclusions

The FORAGE ALFALFA simulation model is a tool that allows dynamic simulation of alfalfa growth under a more diverse climatic and edaphic continuum than the current alfalfa model in DAFOSYM. Simulation of whole plant nitrogen uptake, fixation, and mobilization along with improved biomass production and stand reduction prediction increases model utility in more sophisticated modelling exercises within the Dairy Forage System. Continued validation with variable inputs (both temporal and spatial) are required. Forage ALFALFA is a tool that can be used to study long term effects of crop sequencing, including a forage legume, on environmental quality and crop growth.

Table 1. Validation data for the Forage ALFALFA model, cutting management study Ithaca, New York (1985,1986).

Treatment	Year	Harvest					
		1		2		3	
		Yield	MSW	Yield	MSW	Yield	MSW
	85	4824	4.4	5233	7.4		
Observed	86	6480	5.1	5900	5.7		
	Mean	5652	4.8	5566	6.5		
2-Cut							
	85	4980	4.4	5650	7.7		
Predicted	86	6040	5.5	5320	6.9		
	Mean	5510	4.9	5485	7.3		
	85	4364	4.0	2955	3.9	3114	4.0
Observed	86	5259	3.1	3294	3.1	2824	3.4
	Mean	4812	3.6	3125	3.5	2969	3.7
3-Cut							
	85	3860	2.9	4440	4.7	3420	5.1
Predicted	86	5330	3.6	4330	3.9	3610	4.1
	Mean	4595	3.3	4385	4.3	3515	4.6

MANAGEMENT OF ALFALFA-QUACKGRASS MIXTURES

A.S. DJAJANEGARA, K.A. ALBRECHT and R.P. WALGENBACH

Introduction

Quackgrass is not intentionally introduced into alfalfa fields but is a component of nearly every alfalfa stand in the state. It is considered to be a serious and insidious weed problem in established alfalfa. Quackgrass is aggressive and can outcompete and shorten the stand-life of alfalfa. However, the seriousness of the problem is sometimes not recognized because dry matter yields of alfalfa-quackgrass mixtures are similar to those of pure afalfa stands. Little is known about how specific management practices, or combinations of them, influence alfalfa-quackgrass mixtures. We are investigating the influence of harvest management and potassium fertility on species composition and forage quality of alfalfa-quackgrass mixtures.

Materials and Methods

Alfalfa was established on quackgrass infested sites near Hancock and Prairie du Sac in the late summer of 1988. Beginning in the spring of 1989, four management practices (treatments) were imposed in all combinations to the alfalfa-quackgrass mixtures. The treatments were: 1) grass herbicide (+ or -), 2) summer harvest frequency (three vs. four harvest by late August), 3) autumn harvest (harvest vs. no harvest after October 15, and 4) potassium fertility (0 vs. maintenance levels applied annually). Total forage yield, proportion of alfalfa and quackgrass, and forage quality were determined for each mixture at each harvest.

Results and Discussion

Frequent harvest during the summer (four harvests) resulted in equal forage yield but higher forage quality compared to the three-summer-harvest systems in the first year after establishment (Table 1). In the second year, the four-harvest schedule resulted in lower forage yield although it still maintained higher forage quality compared to the three-harvest schedule. The four-harvest schedule also reduced fall harvest yields and increased quackgrass quality.

The four-harvest schedule resulted in a higher proportion of quackgrass in the mixture. This could have resulted from reduced alfalfa stands or lower alfalfa vigor and growth potential, thus allowing quackgrass an opportunity to invade. If this trend continues, quackgrass invasion may result in the four-harvest system being lower yielding and lower quality than the three harvest system.

Autumn harvest made a significant contribution to seasonal forage yields. Autumn harvests reduced spring yields on the following year and tended to decrease the proportion of alfalfa in the mixtures.

Adequate soil potassium levels helped maintain a greater proportion of alfalfa in the mixture and increased total forage yields compared to low potassium levels. Potassium fertilization also resulted in increased levels of potassium in the tissues of both alfalfa and quackgrass (data not shown).

The presence of quackgrass in the mixture reduced forage quality, but eliminating quackgrass reduced forage yield. Quackgrass always contained greater NDF and lower protein concentrations than alfalfa. Quackgrass in the four-harvest systems was of substantially higher quality than in the three-harvest system.

No consistent interaction among treatments has been found that could lead to identification of the best combination of management practices to minimize the impact of quackgrass in mixtures with alfalfa. Further observation of the established stands is needed to obtain information on long-term effects of each treatment and interactions among treatments.

Table 1. The effect of fall harvest on forage yield, composition, and quality in the first and second year after alfalfa establishment.[#]

	Hancock			Prairie du Sac		
	Fall cut[##]			Fall cut		
	(-)	(+)	LSD	(-)	(+)	LSD
First season						
Yield (Mg/ha)	14.3a	15.33b	0.30	9.80	10.46	1.18
NDF (g/kg)	425a[###]	410b	6.6	381a	365b	8.1
ADF (g/kg)	326a	313b	5.2	289a	271b	6.9
CP (g/kg)	209	212	3.5	211a	219b	5.2
First cut, second season						
Yield (Mg/ha)	3.24a	2.68b	0.18	5.05a	4.52b	0.46
Alfalfa (%)	85.6	80.4	8.2	78.5	75.4	9.1
Quackgrass (%)	14.4	17.1	7.2	14.4	13.1	6.6
Other weed (%)	0.1a	2.5b	0.3	7.0a	12.9b	5.8
NDF (g/kg)	374a	364b	7	415	402	16
ADF (g/kg)	270a	265b	4.5	304	293	10
CP (g/kg)	234	234	4	203	201	6
Second season						
Yield (Mg/ha)	11.68a	12.23b	0.23	11.78	12.29	0.87
Alfalfa (%)	94.5	94.2	2.3	80.3	81.0	10.7
Quackgrass (%)	5.5	5.3	2.2	12.6	7.8	7.6
Other weed (%)	0.0a	0.5b	0.46	7.1	11.2	5.1
NDF (g/kg)	418a	398b	4.1	401a	378b	8.4
ADF (g/kg)	319a	303b	3.9	295a	277b	12.4
CP (g/kg)	216a	221b	2.0	211a	216b	4.2

[#]Composition data is for quackgrass infested plots only.
[##](-)=Without fall harvest; = with fall harvest.
[###]Where significant differences at the 5% probability level exist within fall harvest, values are followed by different letter.

DIRECT ASSESSMENT OF SYMBIOTICALLY FIXED NITROGEN IN THE RHIZOSPHERE OF ALFALFA

M.P. RUSSELLE, D.L. ALLAN and C.J.P. GOURLEY

Introduction

Effectively nodulated legumes provide large amounts of N to cropping systems. The widely grown forage legume, alfalfa, fixes about one million metric tons of N each year in the eight USA Corn Belt states. Much of this fixed N is harvested in herbage and is fed to animals, but evidence from a range of experimental methodologies confirms that alfalfa and other legumes can deposit significant amounts of N in the soil during growth. Both classical and contemporary research estimated that net N accretions of about 56 kg N/ha occur annually during alfalfa growth. Probably twice this amount is actually contributed by alfalfa because soil N accumulation and removal in harvested herbage is in the range of 40 to 80 kg/ha per year. In additon, annual amounts of symbiotically fixed N transfer from alfalfa to associated grasses have been estimated to be as much as 20 kg N/ha. What are the important pathways through which this symbiotically fixed N enters the soil?

Potential pathways of N loss from legumes include: excretion of N from roots and nodules; senescence and degradation of nodule and root tissue; direct interconnection of grass and legume roots via mycorrhizal fungi; ammonia loss from legume herbage and reabsorption by grass herbage; and movement of N from legume herbage to the soil via leaching or decomposition of surface litter. Pathways through mycorrhizal fungi and gaseous transfer are not likely to explain the large accretion of soil N under legumes. The major pathway(s) of N loss must be determined to improve internal recycling of symbiotically fixed N in cropping systems that include legumes. Losses of this and other sources of N must be minimized to reduce potential environmental degradation.

Losses of nitrogenous substances from roots of growing alfalfa appear to be small (1 to 4.5% of total symbiotically fixed N) underhydroponic, gnotobiotic, and soil conditions. There are no reports in which N deposition was measured directly under conditions that simulate the field environment. The objective of this experiment was to directly quantify the net amount of symbiotically fixed N in the rhizosphere of established alfalfa.

Materials and Methods

Separate batches of soil were obtained from the Ap and B2/C1 horizons of a Hubbard loamy sand soil (Udorthentic Haploboroll). The soil was screened (1.2-cm mesh) to remove rocks and larger plant residues, limed and fertilized according to Univ. of Minnesota recommendations, and packed to approximately 1.27 g/cm^3 dry bulk density into 15-cm diameter by 1-m long PVC tubes, with 30 cm of Ap soil over 70 cm subsoil. The PVC tubes had been closed on the bottom end with PVC caps, fitted with glass wool as a filter and tapped to allow collection of leachate. All drainage lysimeters were placed in an insulated, air-conditioned enclosure with soil surfaces level with the top of the enclosure.

Three genotypes of alfalfa were used in this experiment: Saranac, a widely grown commercially-available cultivar; In-Saranac, a host-mediated ineffectively nodulated line of alfalfa; and an unnamed line of alfalfa selected for apparent N excretion. All genotypes were grown from seed in a sand bench for 10 weeks before transplanting four plants of each to separate lysimeters. Plants received water, nutrient solution, and supplemental lighting, and were harvested four times before initiating labeling. During the three later harvests, successive replicates were harvested every other day to establish the staggered intervals needed during labeling and sampling. Labeling with $^{30}N_2$ began for the first replicate on 2 January 1989. Saran bags were sealed to the tops of each lysimeter in the replicate after connecting a gas circulating system that connected the treatments in parallel. The system was tested for leaks, some of the gas was removed, and a mixture of $^{30}N_2$-Ar-O_2 was added to achieve an average ^{15}N concentration of about 5 atom percent in N^2, Ar, and O_2 concentrations of about 60, 18, and 21%, respectively. The atmosphere was circulated intermittently through the three lysimeters for 48 hours and gas samples were obtained periodically for isotope analysis. Calculation of N_2 fixation was based on the time-weighted average ^{15}N concentration of the atmosphere for each replicate.

After 2 days of labeling, herbage was harvested from the replicate and the gas system was moved to the next replicate. After two days for "equilibration", the roots and soil were carefully separated from six replicates; the remaining six were allowed to regrow for about 5 weeks before harvesting herbage and sampling roots and soil. Rhizosphere soil was defined as that soil adhering to the roots upon removal from the other "bulk" soil and was washed from the roots by brief sonication in 0.08 M phosphate buffer, pH 7.0. After dry mass determination, samples were finely ground and analyzed for total N, total C, and ^{15}N by Dumas combustion and mass spectrometry.

Because roots were not entirely free of soil, contamination was estimated based on the expected C concentration of roots (446 mg/g) and the observed C concentration of the rhizosphere soil. Root N concentration was increased based on calculated soil contamination.

Results and Discussion

Generally, Saranac was not different from the "excreting" line in yield or N concentration of any plant components, but the ineffective line was significantly lower in all cases. Assuming that N^2 fixation patterns follow those shown by other researchers, extrapolated N^2 fixation rates of the Saranac and the "excreting" line were similar to rates others have measured in the field.

Total soil N content increased in the rhizosphere, compared to the bulk soil. This increase amounted to the equivalent of about 20 kg/ha for the effective lines and about 9 kg/ha for the ineffective line. Although there was significant accretion of total N in rhizosphere compared to bulk soil, little (less than 2% of total N fixed by the effective plants) was derived from newly fixed N and there were no differences between the "excreting" line and Saranac.

The results of this research are consistent with the findings of two other experiments using the indirect ^{15}N isotope dilution technique (reported in the 1988 Research Summary). Together, these experiments provide evidence that net N accumulation in the soil during alfalfa growth is likely due to other processes, such as decomposition of plant parts, rather than to N excretion from roots and nodules. Root and nodule senescence of alfalfa and birdsfoot trefoil are presently under investigation.

NITROGEN AND DRY MATTER CONTRIBUTION OF NONHARVESTED FORAGE LEGUMES

M.H. HALL, C.C. SHEAFFER, G.H. HEICHEL, M.P. RUSSELLE and F.E. THICKE

Introduction

In government cropland diversion programs, land may be diverted from grain crop production for one or more years and forage legumes may be grown. Forage legumes grown on diverted cropland cannot normally be harvested or grazed except during emergency feed shortages, such as the one that occurred during the 1988 drought. When forage legumes are not harvested, crop dry matter (DM) and N accumulate until incorporation into the soil before grain crop production resumes. The yield response of corn following alfalfa and other forage legumes grown in conventional rotations in which the forage is periodically harvested has been the subject of much research. Legumes stimulate the yield of subsequent nonlegume crops through addition of N and through non-N "rotation" effects.

There is a paucity of information on corn yield response to incorporation of nonharvested forage legumes managed as prescribed for diverted cropland. Growers need to know the yield response of corn following incorporation of nonharvested legumes when land is released from diversion. Our objectives were to determine: 1) DM and N yield of 1- to 3-year-old stands of alfalfa, red clover, and birdsfoot trefoil when stockpiled *in situ* as prescribed for diverted acres and when subjected to a conventional three harvest management; and 2) the impact of these legumes and harvest regimes on soil nitrate quantity and DM and N yield of subsequent corn crops.

Materials and Methods

A field experiment was conducted from 1982 to 1985 on a deep Tallula silt loam

soil (Typic Hapludoll) at the Rosemount Agricultural Exp. Stn., Rosemount, Minnesota, in which these three legume species were each grown for 1, 2, or 3 years and were subjected to two harvest regimes as described below. In October of each year, legume herbage and root residues were moldboard plowed and in subsequent years corn was grown. Legumes were managed by one of two harvest regimes: 1) conventional, in which legumes were harvested twice during the seeding year (year 1) and three times at one-tenth bloom before 15 September in subsequent years; and 2) stockpiled, in which the legume forage was not harvested, but was stockpiled *in situ* for 1, 2, or 3 years. Legume herbage yields were determined at each harvest for the conventional treatment and in fall before plowing for both treatments. Crown and root yields were determined by undercutting the plots at about 8 inches before plowing. Soil samples were taken each spring in 1-foot increments to 5 feet and analyzed for nitrate N concentration and bulk density. Corn was planted in April of each year and grain and total DM yields and N accumulation were determined at physiological maturity. Plant sample N concentration was determined with Kjeldahl digestion, followed by steam distillation.

Results and Discussion

Over all species and years, more DM (285%) and N (244%) were available for incorporation in fall from stockpiled than from conventionally harvested legumes. This is attributable to greater herbage accumulation because root plus crown DM and N accumulation was about 15% less with stockpiling. Across years, stockpiled alfalfa and birdsfoot trefoil accumulated 280% more DM than red clover, but similar amounts of N. In contrast, conventionally harvested birdsfoot trefoil provided about 50% less DM and N for plowdown within and across years than did similarly managed alfalfa and red clover.

Averaged over all years, soil nitrate in the top foot of soil was greater after stockpiling (43 lb/acre) than after harvesting (32 lb/acre). Stockpiling of legumes resulted in a 20 lb/acre increase in total soil nitrate to the 5-foot depth. Soil nitrate in spring did not correlate with the amount of legume N plowed down the previous fall. Nitrogen losses from roots and nodules, differences in root production beneath the depth of measurement, leaching of nitrate into the soil profile from senesced stockpiled herbage, and differences in soil N use by the legumes may have affected residual nitrate concentrations.

Corn stover and grain DM yields in any year were not consistently affected by legume species and an interaction between species and harvest management was not evident. Legume harvest management in years 1 and 2 of production did not affect subsequent corn yields, but, following 3 years of legume production, corn yield was greater following stockpiled than harvested legumes (7.9 vs 7.0 tons total DM/acre).

In contrast to DM yields, corn N accumulation was greater following as little as 1 year of stockpiling compared to conventional harvesting. Nitrogen accumulation in the total corn plant after 3 years of legume stockpiling was 51% greater than following harvesting. To examine the effect of legume N incorporation on corn N accumulation, we combined data for first-year corn over the 3 years of the experiment after normalizing N accumulation to the maximum observed each year. The relative amount of N accumulated by corn during the first year after plowdown was related (R^2=0.78) to the amount of herbage N incorporated, but was not as well related to total (herbage+roots and crowns) N plowed down (R^2=0.61). Nitrogen accumulation by corn, when very small amounts of legume herbage N were incorporated, ranged from about 68 to 107 lb/acre and was about 60% of the maximum observed N accumulation. Maximum corn N accumulation was achieved at legume herbage N additions between 125 and 150 lb/acre. Standing herbage of stockpiled legumes generally contained at

least 125 lb N/acre by the end of the second year after seeding.

Producers can expect that stockpiling forages, as occurs in government programs, will consistently result in greater amounts of DM and N for incorporation than conventionally harvested treatments. For production of DM under stockpiling, we recommend use of alfalfa or birdsfoot trefoil. Stockpiling of legume forages should lead to an enhancement of soil organic matter concentrations and soil N status. However, because of large amounts of N and organic matter available in the soil pool, these additions may not result in enhanced crop yields. We found that despite increases in soil N and corn N uptake each year due to stockpiling, significant increases in corn yields were not observed until after 3 years of stockpiling.

DIFFERENCES IN NITROGEN UPTAKE OVER TIME AND DEPTH AND ORGANIZATION OF AN OLD-FIELD PLANT COMMUNITY

R.B.McKANE, D.F. GRIGAL and M.P. RUSSELLE

Introduction

The question of how plant species can coexist on sites with limited resources (nitrogen, water, CO^2, etc.) has been the subject of research over many decades. The majority of this research has been conducted by ecologists studying natural or disturbed sytems, but the question has direct relevance to mixed species plantings (in pasture, for example) or in mixtures of crops and weeds.

For animals, equilibrium theories state that the number of species is restricted to the number of limiting resources. This cannot be applied to plant communities because most environments are limiting in three or four resources and species diversity is generally much greater. Although some have suggested that this species diversity observed in many plant communities can only result from nonequilibrium, others assert that spatial and temporal partitioning of the resources allow several plant species to coexist on a few limiting resources. There is abundant evidence that co-occurring plant species often differ in rooting depth, phenology, or both depth and time of root activity. Previous experiments have not demonstrated whether such differences allow co-occurring species to sufficiently subdivide specific limiting resources.

In this experiment, we tested the hypothesis that spatial and temporal differences in soil N use facilitated species' coexistence in an old-field plant community.

Materials and Methods

The experiment was conducted on a 50-year-old abandoned agricultural field at the Cedar Creek Natural History Area in Minnesota. Sixty-eight plant species occur within the field, but the six most common species account for about 85% of the total above ground biomass. The soil is a well drained fine sand with low total N concentration (170 g/m^2 in the upper 20 cm). Available N is the most limiting primary resource and mineralization of soil N shows distinct time and depth differences.

We injected ^{15}N at two depths (2 and 12 cm) at each of three times (1 May, 25 June, and 10 August) to label the available N pool. Injections were made on 10-cm spacings in separate 3 by 3 m plots. Total N applied by injection was 0.5 g/m^2. Plant samples were collected about 50 days after injection, resulting in early-, mid-, and late-season N uptake intervals. Only the central 2 by 2 m area of each plot was harvested. About 10 plants of each of the six most common species were sampled within each of 16 quadrats in the $4m^2$

central area. Plants were oven-dried, ground, and digested by a modified semimicro Kjeldahl procedure that included nitrite and nitrate reduction to ammonium. Samples were steam distilled for N quantification and were analyzed by mass spectrometry for isotope concentration. Isotope accumulation was calculated and then expressed as a percent of total ^{15}N uptake over the three seasons and two depths.

Results and Discussion

Each of the six species showed significant differences in time and depth of ^{15}N uptake. *Poa pratensis* and *Panicum oligosanthes* were characterized by high early-season uptake (between 52 and 60% of the uptake total) and greater uptake from 12 cm than from 2 cm. *Artemisia ludoviciana* and *Solidago nemoralis* also had high early-season uptake (between 54 and 60% of the uptake total), but depended more on N at the 2-cm depth than *Poa* and *Panicum*. In contrast, *Schizachyrium scoparium* and *Ambrosia coronopifolia* showed high N uptake during mid-season (between 53 and 55% of the uptake total).

Differences in temporal patterns of N accumulation in *Poa* and *Schizachyrium* are likely based in their respective C_3 and C_4 growth habits. For all species, N uptake was closely related to their phenology. Discriminant analysis helped separate the species into logical groupings, or "niches" (Fig. 1). The six treatment variables were reduced to two orthogonal (perpendicular) multivariate axes; that is, the horizontal axis was correlated with ^{15}N uptake during early-season at 2-cm, mid-season at 2-cm, and late-season at 12-cm, whereas the vertical axis was correlated with the other three combinations of treatments. The horizontal axis explained 70% of the variance among species and separates "early-season" species (*Poa, Panicum, Artemisia,* and *Solidago*) and "mid-season" species. The vertical axis explained an additional 24% of the variance among species and primarily separated the "early-season" species into two groups according to their dependence on mid- vs. late-season uptake and on uptake from 2 cm.

The relative abundance of each species also is shown in Fig. 1. When considered together with the separation of species in the two dimensional niche space of the graph, it is clear that spatiotemporal differences in N uptake is a major determinant of community organization. The two most dominant species (*Poa* and *Schizachyrium)* are well separated in niche space, low-ranking subordinate species (*Ambrosia* and *Panicum*) occupy niches similar to the dominants, and high-ranking subordinates (*Artemisia* and *Solidago*) show better separation from the dominant species than do low-ranking subordinates. These observations support the hypothesis that competitive interactions between

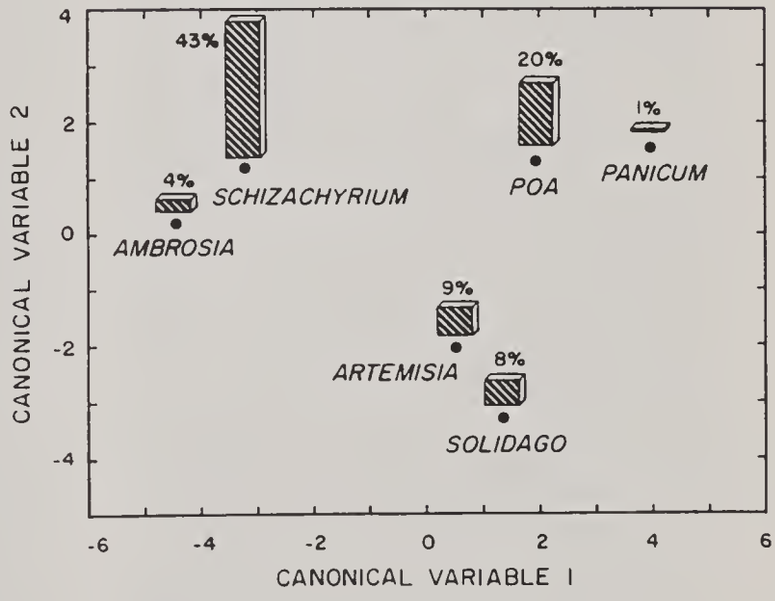


Figure 1. Discriminant analysis of patterns of ^{15}N uptake in a 50-year-old abandoned agricultural field. The multivariate axes accounted for 94% of the total variance among species. The columns and percentages adjacent to them represent each species' proportion of total (all species) peak above ground biomass.

dominant species exert a powerful control on the abundance of other species, and that the intensity of such interactions are related to differences in specialization. It appears that differentiation is more important among dominant than subordinate species. To the extent that equilibria exist between dominant and rare species, more than one rare species could occupy the same niche for long periods because there is a low probability of rare species interacting as neighbors.

Spatial and temporal partitioning of a single limiting resource is significant among co-occurring plant species in this old-field. This partitioning may reduce interspecific competition and thereby facilitate coexistence. Other factors besides spatiotemporal resource partitioning contribute to species diversity at this and other sites, including variations in N availability over the landscape, differences in maximal growth rates of early- vs. late-successional species, small-scale disturbance due to gophers, and selective consumption of species (e.g., legumes) by herbivores. The importance of resource partitioning is sufficient, however, to explain most of the variation in N uptake among species.

SOIL N MINERALIZATION INDEXES TO PREDICT CORN RESPONSE IN CROP ROTATIONS

F.E. THICKE, M.P. RUSSELLE, O.B. HESTERMAN
and C.C. SHEAFFER

Introduction

Due to economic and environmental concerns, interest has been renewed in developing improved methods for assessing soil N mineralization potential. Past efforts to identify a useful laboratory N mineralization index have been hampered by many factors including variability in weather, variability in the amount of residual inorganic N present from previous N fertilization, influence of different cropping systems on N management, and the legacy of inexpensive N fertilizer. There are two classes of soil N mineralization indexes. Biological incubation methods provide a valid relative measure of soil N mineralization potential but are impractical for routine use because of the time required for incubation. Chemical indexes are preferable because they are generally more rapid, convenient, and precise and are less likely to be affected by sample handling and storage. However, chemical indexes are empirical and are unlikely to selectively release the same fraction of N that would be released by the soil mineralization-immobilization cycle. Much of the N released during mineralization comes from labile reservoirs, including plant residues. Soil samples for N mineralization studies are conventionally collected in a manner that excludes crop residues. This may preclude the possibility of accurately assessing the N mineralization potential of soils containing significant amounts of residue. The objective of this experiment was to formulate and validate empirical predictive models of dry matter (DM) and total N accumulation of corn grown in rotation with other crops, based on soil inorganic N concentration, applied N rate, various soil N mineralization indexes of soil containing crop residues, and previous crop.

Materials and Methods

A crop rotation study was conducted at four Experiment Station locations in Minnesota during 1982 and 1983. The soils included an irrigated sandy loam, one rainfed silt loam, and two rainfed clay loams. Alfalfa, corn, fallow, N_2-fixing soybean, non-N_2-fixing soybean (at two locations), and wheat (at two locations)

were established in 1982. Each of two genotypes of alfalfa, 'Saranac AR' and 'MN Root N' (a nondormant synthetic selected for high root N concentration and large root and herbage mass) were subjected to two cutting managements, either the conventional 3 harvests or only one (in July) at one-tenth bloom. Each location had 7 crop-management combinations plus fallow treatments. Wheat, corn, and soybeans were harvested for grain. All plots were moldboard plowed in fall 1989. In 1983, all plots were planted to corn with 0.76-cm row spacings, and ammonium sulfate was applied to subplots of all main plots at five rates (0 to 224 kg N/ha, except 0 to 135 kg N/ha at the driest location, Lamberton). Corn was harvested at physiological maturity and DM and N accumulation were determined.

Soil samples were obtained shortly before planting from the plow layer with a specially-designed coring device which cut through and included crop residues that had been plowed down. Separate samples were taken with a hydraulic probe in 30-cm increments to 150 cm (90 cm at one location because of a restricting layer at that depth) with a hydraulic probe. All soils were air dried and ground to pass a 2-mm screen. Inorganic forms of N and several N mineralization indexes were obtained for each plow layer sample; only inorganic N forms were determined on the deep samples. Mineralization indexes included aerobic incubation, anaerobic incubation, autoclave extraction, acid permanganate extraction, and glucose extraction. Each had been shown to correlate well with other indexes or with crop response under controlled conditions.

Correlation analysis was used to identify whether the relationship between soil profile nitrate-N and nonfertilized corn grain yield or total N uptake was significant. Predictive models of corn grain yield and N uptake were produced using forward stepwise regression technique with SAS. Independent variables included all soil measurements and "dummy" variables representing the previous crops. Models were validated using data from three independent experiments conducted in 1984 and 1985 (three location/year combinations).

Results and Discussion

Corn experienced a range of weather-related stress during 1983 making yield response to previous crop treatment variable. Use of coefficients relating to previous crops explained less than one-half the variability in nonfertilized corn grain yield and total N uptake. In contrast to this approach, there has been increased empasis on use of soil nitrate concentrations in the uppermost 60 cm as an index of N availability. Soil nitrate concentrations in the upper 30 to 60 cm were not well correlated with grain yield (r=0.35) but had higher correlations with total N accumulation (r=0.72). The use of soil nitrate availability throughout the rooting profile (i.e., to 120 or 150 cm) is currently recommended in the driest location, and several researchers have proposed using this approach in more humid regions. Using this approach, with estimated N availability from the previous crop and N mineralized from soil organic matter resulted in much better predictions of corn grain yield (r=0.76) than using topsoil nitrate concentrations alone. However, this approach could explain only one-half of the actual fertilizer N requirement to attain maximum yield.

No N mineralization index was consistently related to corn grain yield or total N accumulation except for the results of one week of aerobic incubation. We used this index as an independent variable in the stepwise regressions. Across locations, about 65% of the variability in nonfertilized corn grain yield was explained by inorganic N concentrations in the plow-layer, 74% was explained after adding the one-week incubation results, and 82% was explained after adding adjustments for previous fallow or wheat. For total N accumulation in mature corn, these R^2 values were 73, 81, and 87%, respectively, with the last including adjustments for previous soybean and wheat. These equations explained a surprising amount

of the variability in measured yield and N accumulation considering the diverse soils and weather experienced by the corn crop.

Despite the development of promising regression equations from the initial experiment, validation tests of these equations with the three independent site/years of data indicated that the original equations could explain no more that one-half the variability in observed grain yield and N accumulation. It is possible that other variables need to be included in the equations or that a larger array of data needs to be included in the development of such equations. It may not be possible to precisely predict N availability and crop response given the dynamic nature of the N cycle and the variability imposed by the interaction of weather, crop management, and crop growth on the N cycle. Nevertheless, accounting for inorganic N in the soil at the beginning of crop growth and estimating N release from organic forms of soil N and plant residue N should provide the best chance for achieving accurate prediction of expected yields and fertilizer N needs.

RAPID PROTON NMR METHOD FOR DETERMINATION OF *threo:erythro* RATIOS IN LIGNIN MODEL COMPOUNDS

J. RALPH and R.F. HELM

Introduction

Lignin model compounds are used extensively to model aspects of lignin in plant cell walls. Using model compounds it is possible to obtain accurate NMR data relevant to the lignin polymer, to examine reactivity, and to explore cross-linking of lignins with carbohydrates and phenolic acids. The stereochemistry of lignin model compounds has an important impact on their reactivity and affects the three dimensional shape of the molecules. To date the methods for determining this stereochemistry are relatively straightforward but time consuming. Quantitative ^{13}C NMR is non-destructive but requires at least 20 mg of compound to perform the analysis in a timely fashion. The most common method is to acetylate the sample and run proton-NMR spectra. The acetylation, while not difficult, requires close to 1 hr of operator time for reaction and work-up. Also, acetylations are often performed overnight so the results may not be obtained for a day. We recently proposed a sensitive method using trifluoroacetate derivatives which can be made directly in the NMR tube. Proton NMR can be used but is not satisfactory for etherified models. Analysis by ^{19}F NMR is very sensitive and diagnostic, but the general inaccessibility to NMR instruments equipped for ^{19}F observation has precluded its widespread use. The main problem with both the acetylation method and the trifluoroacetylation method is that relatively high field-strength instruments are required and the sample has to be derivatized. We report here on a very simple and sensitive proton NMR method that can be used effectively at lower field strengths without the need for sample derivatization. The dispersion between the threo and erythro isomer resonances is also greater than for the previous methods.

Method

The method is straightforward and requires only slight modification of the way any NMR spectrum would be run with acetone-d_6 as solvent. The model, 1-5 mg, or less if sample is limited, is dissolved in acetone-d_6 in a 5 mm NMR tube. A proton NMR spectrum is run in the normal way (with little regard to pulse width or relaxation delays) and the narrow α-OH proton doublets near δ 4.5 are integrated. For free-phenolic models, the phenolic OH protons near δ 7.5 should also be integrated. If the α-OH proton doublets are not evident in the spectrum, 1 to 2 µl of H_2O are added and the spectrum is re-run.

Results and Discussion

Acetone-d_6 is a commonly used solvent for NMR spectroscopy, second only in usage to $CDCl_3$. It has a sharper lock signal and is therefore a superior solvent for high resolution work. For some reason the appearance, Figure 1, of proton spectra of β-ether lignin model compounds in this solvent has escaped comment in the literature. At first sight the alpha proton at about 4.9 ppm, normally a clean doublet (coupled to the beta proton), appears complex, and extra peaks are evident in the region between the methoxyl protons and Hα. Closer inspection shows these excess doublets to be the OH protons on the α-hydroxyl group. In acetone they do not exchange in the NMR time-frame as they do in $CDCl_3$ and therefore appear as sharp doublets (coupled to the α-protons). Similar coupling of the proton on the γ-

OH group also complicates the splitting patterns of the Cγ protons. So for characterizing the compound of interest, acetone is a poor choice for proton NMR as it leads to considerable complication of the spectra. However, in acetone-d_6, the *threo* and *erythro* α-OH protons are sharp and extremely well resolved, so this solvent is an excellent choice for determining isomer ratios. For free-phenolic models, the phenolic OH protons near δ 7.5 are also resolved and can also be used (Figure 1). The NMR field strength is no longer a crucial factor because of the excellent dispersion of the critical signals, typically 0.12 ppm for the α-OH protons and 0.03 ppm for the phenolic-OH protons.

Conclusion

With the *threo:erythro* ratio determination method described above, it is straightforward to determine these ratios on small quantities of compound without the need for derivatization. Samples prepared for NMR in this way are also excellent for full assignment and connectivity analysis using COSY and relayed coherence transfer COSY experiments, and should be equally valuable for TOCSY (HOHOHA), NOESY and various C-H correlation experiments. As the stereochemistry of a lignin model is often of critical importance, we hope that this will be reported now that a simplified method for stereoisomer determination is available.

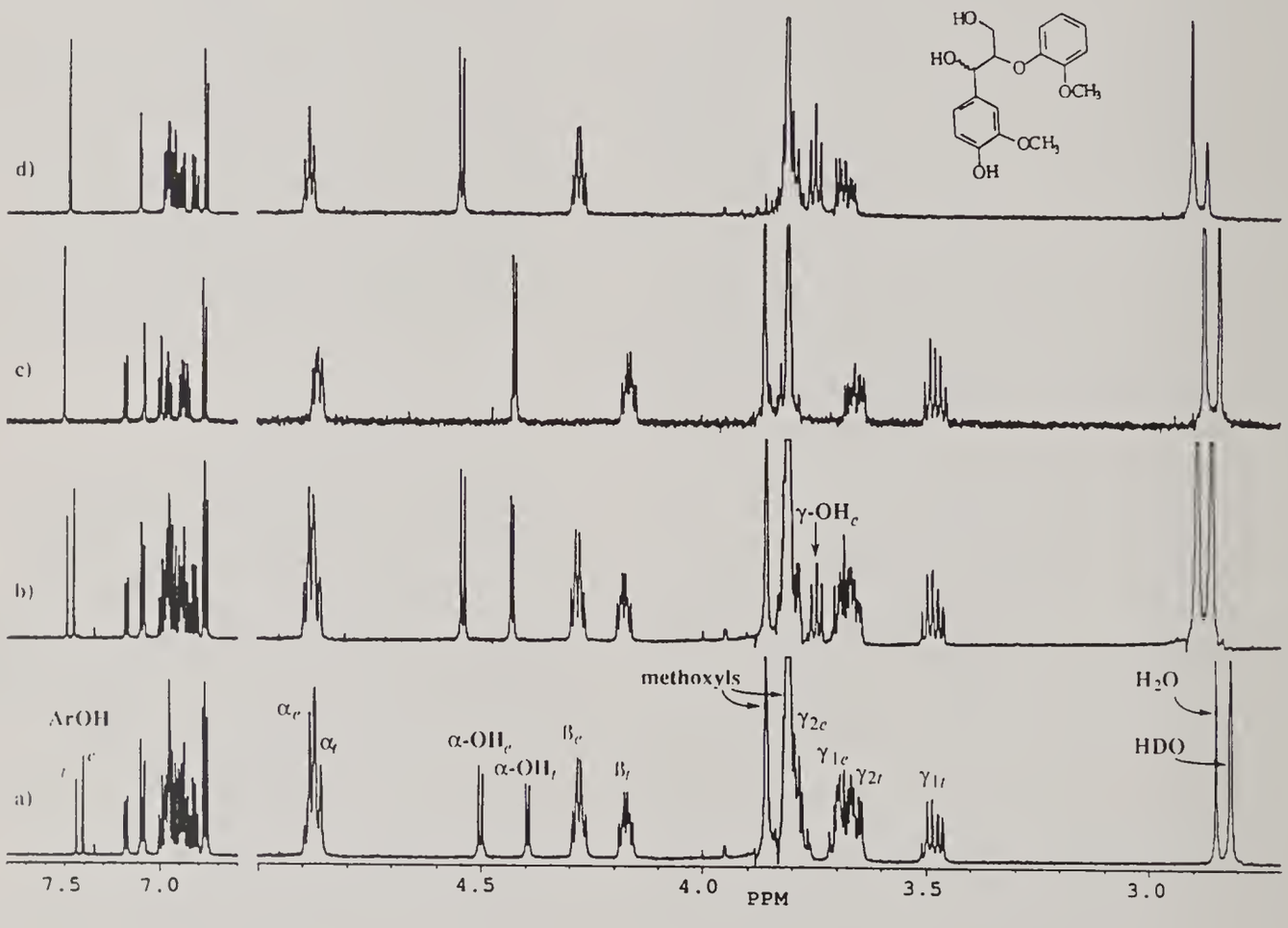


Figure 1: Proton NMR spectra (500 MHz) of guaiacylglycerol-β-guaiacyl ether. Note that the aromatic and phenolic region is on a different horizontal and vertical scale that the aliphatic region in these plots. a) threo + erythro (t<e). b) same as a) but with 1 μl H_2O added. Note the increased H_2O peak , the increased intensity of the phenolic and α–OH peaks and the migration of all OH peaks. c) threo isomer. d) erythro isomer.

THE STEREOCHEMISTRY OF GUAIACYL LIGNIN MODEL QUINONE METHIDES

J. RALPH

Introduction

Quinone methides are reactive intermediates that are important in the lignification process. Their structure, stereochemistry and reactivity dictate their subsequent reactions in building up the complex cell wall. The recent literature has addressed itself to an apparent discrepancy between experimental and computational results with respect to the conformation of a series of quinone methide compounds. Based on experimental results, we have been reported that, from both proton and ^{13}C NMR shielding data and nuclear Overhauser effect data, a mixture of *syn* and *anti* (or *Z*- and *E*-) forms of guaiacyl quinone methides are present. We have further indicated that these isomers are non-interconverting and have demonstrated the differential reactivity of the two isomers toward nucleophilic attack. However, a theoretical study by Jakobsons and Shevchenko indicated that, due to low rotational barriers, a dynamic equilibrium between the rotamers would be established, i.e. that the two forms represented not geometric isomers but merely rotational conformers. Their abstract states: "*In the p–quinonemethide series, the barriers of internal rotation are sufficiently low for the realization of the dynamic equilibrium between the conformers in solutions at room temperature. The earlier proposed (Ralph and Adams) interpretation of the nuclear Overhauser effect for one of the derivatives seems to be rather groundless. According to the conformational analysis data, the Overhauser effect is related to a mixture of conformers. The stereoisomeric Z forms of p-quinonemethides were energetically preferred to the corresponding E-forms.*" This paper reports further NMR experiments and our own molecular modelling data which substantiates our original claim.

Methods

Two-dimensional NOESY NMR spectra of the α-deuterated analogues of compounds **1-3**, freeze-thaw degassed and sealed into 5 mm NMR tubes, were run on a Bruker AM-500 500 MHz spectrometer. The computational examination of these structures was performed using the integrated graphics/computational chemistry packages Alchemy II and "SYBYL", licensed from Tripos Associates running on a MacIntosh™ IIx computer and Sun1+ WorkStation respectively.

Results and Discussion

The claim of free rotation and the comments regarding nuclear Overhauser enhancements by Jakobsons and Shevchenko simply overlook the indisputable fact that these isomers are clearly not interconverting on the proton NMR timescale (which, at around 300 MHz, can resolve rotations in the 75 μs to 200 ms range, corresponding to rates of 5 to 10,000 s^{-1} and activation energies of 5 to 25 $kcal.mole^{-1}$. This is clear because the quinone methide spectra (see the high-resolution projections in Figure 1) are a superposition of two, non-interconverting isomers. If they interconverted rapidly on the NMR timescale, only a single 'compound' or averaged conformer would be visible in the spectra. If the rotation was occurring on the NMR timescale, the peaks for each isomer would be broadened and, at some temperature close to ambient, would show coalescent behavior typical of hindered rotation. The fact that the peaks are sharp and well resolved simply refutes any claim of free rotation at reasonable rates.

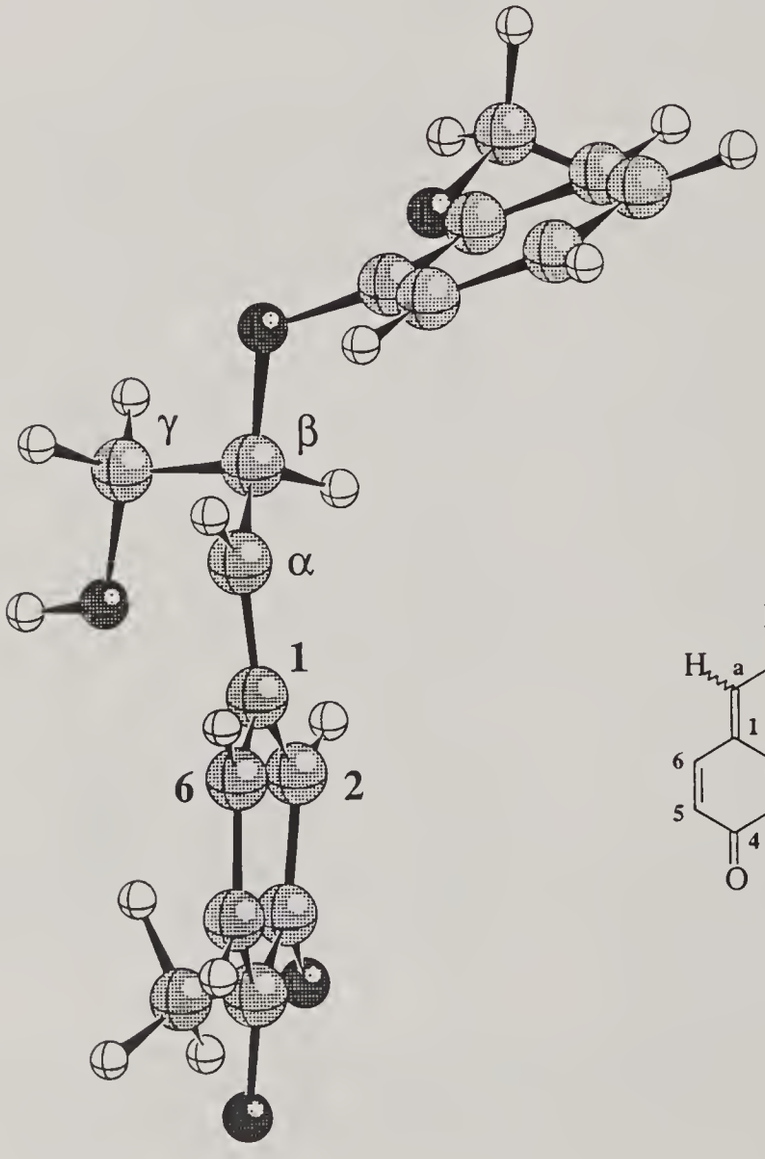
From the 2D NOESY spectra in Figure 1, it is abundantly clear that, for all three quinone methides, H_β-syn correlates with

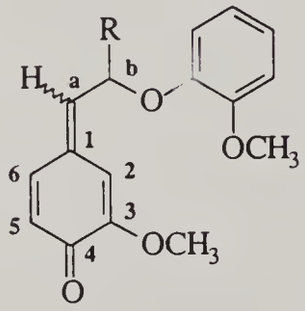
H_2-syn and not H_6-anti, whereas H_β-anti correlates with H_6-anti and not H_2-syn. Thus again, on the NMR timescale, there can be no interconversion of H_β-syn and H_β-anti, i.e. no rotation about the C_1-C_α bond.

Our molecular modelling data, which predict a rotational barrier of 48 kcal/mole, coupled with the NMR chemical shift data, allow prediction of the temperatures necessary to observe peak coalescence and, hence, rotation on the NMR timescale, and prediction of that rate. In quinone methide **1**, even for the most closely spaced peaks, H_5, which would coalesce at the lowest temperature, coalescence would not occur until about 600°C and with a rotational rate of only 30 s^{-1}. Other protons would coalesce at higher temperatures and exhibit correspondingly higher rotation rates at those temperatures. The calculated rotational rate at room temperature is $<10^{-22}$ s^{-1}.

Conclusion

Both experimental data and our theoretical modelling data show conclusively that guaiacyl quinone methides exist as non-interconverting isomers at room temperature and predict that the same holds true at elevated temperatures.





1. R = CH_2OH
2. R = CH_3
3. R = H

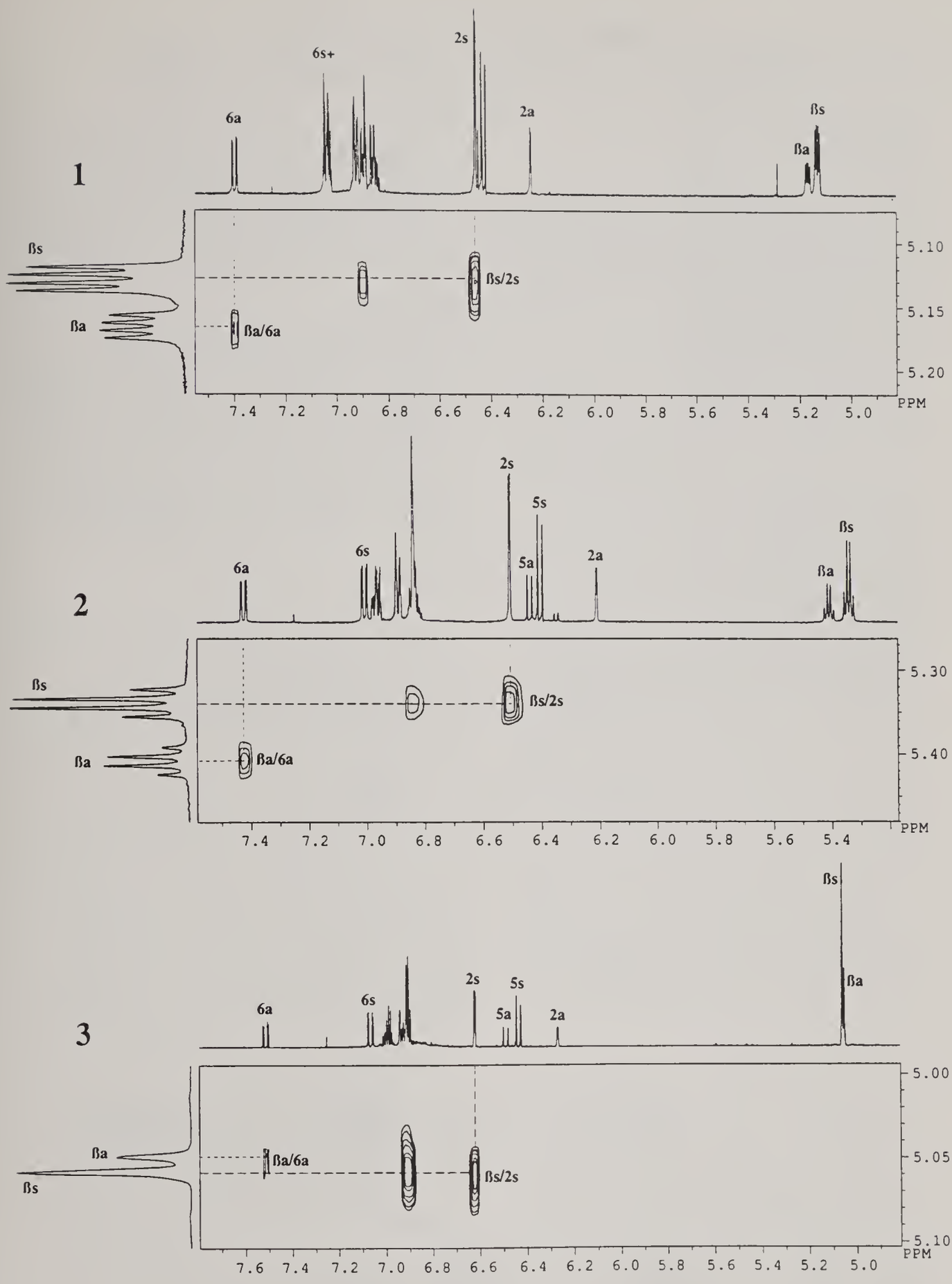


*Figure 1. Partial 2D phase-sensitive NOESY spectra of quinone methides α-D-**1**, α-D-2, and α-D-3, showing the correlation of the β-protons. s= syn (Z), a= anti (E). Spectra of **1** and **2** were taken at 600 MHz, 3 at 500 MHz. Projections show the high-resolution spectra.*

NMR of Lignin Model Trimers

or Why you will never find Crystalline Regions in Lignin!

J. RALPH

Introduction

The use of trimeric lignin model compounds is a natural extension toward more complex and accurate modelling of lignin for structural and reactivity studies of plant cell wall components. In the NMR spectra of dimers such as guaiacylglycerol-β-guaiacyl ether (GGβGE), it has long been recognized that, while the modelling of side-chain and free-phenolic end of the molecule is quite accurate and gives chemical shifts which are in good agreement with higher oligomers and the lignin polymer itself, the B-ring is only poorly modelled since it possesses no side-chain. Trimeric compounds allow accurate modelling of the free-phenolic end and the internal unit. Analysis of their spectra point to some amazing complications that have been overlooked to date. Although most researchers do not consider that lignin can or would contain crystalline regions, this theme arises time and again in publications and at conferences. We will provide compelling reasons to never undertake the futile exercise of looking for them!

Methods

Proton NMR spectra of acetone-d_6 solutions of compounds **1-4** (typically 5 mg of sample in ca. 0.3 ml of solvent in a 5 mm NMR tube) were determined at 25-30°C with a Bruker AM-600 600 MHz instrument. Assignments were made from 2D COSY, 2-step relayed coherence transfer COSY, and J-resolved experiments.

Results and Discussion

Figure 1 shows selected regions of the 600 MHz 1D spectra of the acetates of guaiacylglycerol-β-guaiacyl ether **1**, veratrylglycerol-β-guaiacyl ether **2**, the

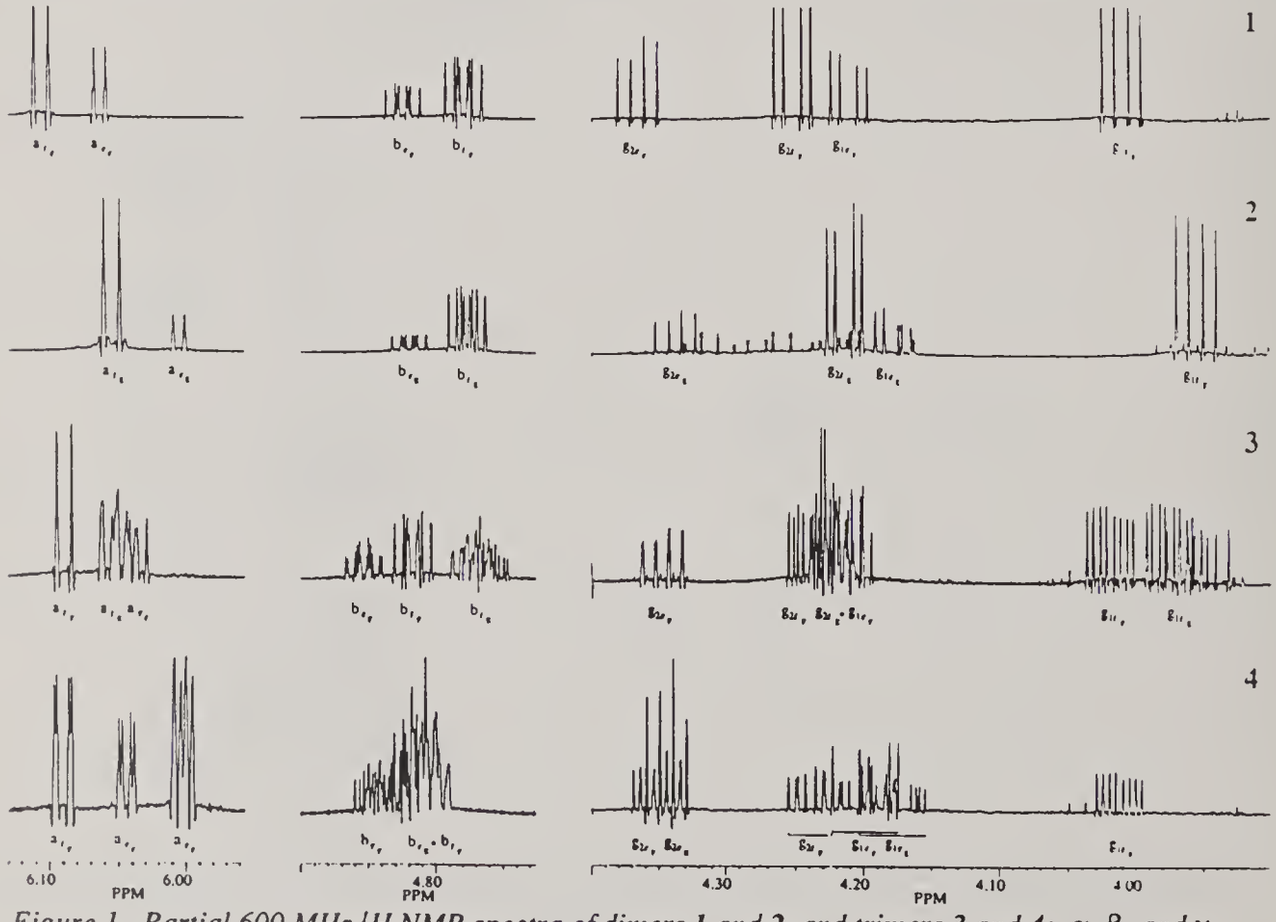
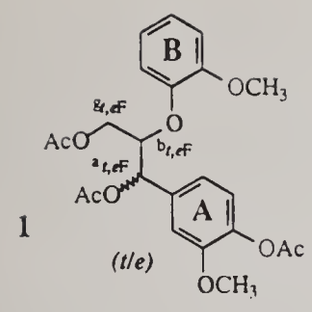
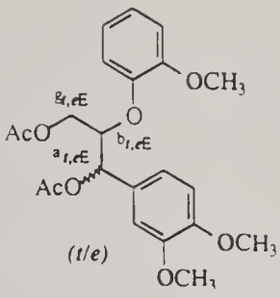


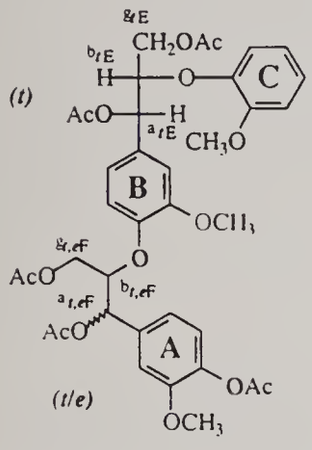
*Figure 1. Partial 600 MHz ^1H NMR spectra of dimers **1** and **2**, and trimers **3** and **4**; α, β, and γ regions.*



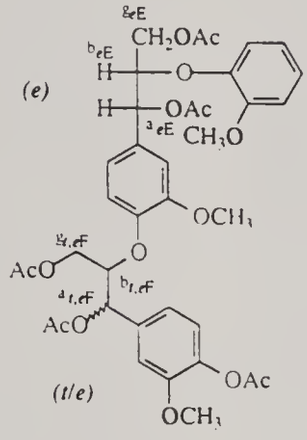
1



2



3



4

t/e-t trimer **3** and the t/e-e trimer **4**. The following conventions are used to identify resonances. In describing a proton, the first letter or letter pair refers to the atom, i.e. α, β, γ_1 or γ_2 for each of the four protons on the side-chain. The second term refers to the isomer, either *t* or *e* for *threo* or *erythro*, and the final term, a capital letter, refers to the position of the unit within the structure, E for etherified (internal unit), F for free (terminal free-phenolic end). Thus α_{eF} refers to an alpha proton, in a unit with *erythro* stereochemistry, at the terminal or free-phenolic end of the molecule.

The resolution and dispersion of the resonances clearly establishes the presence of all expected isomers. For example, the 4 sets of four peaks (doublet of doublets) for the γ_{1tE} (1 set for each of the four possible isomers) is shown in the expansion in Figure 2.

It becomes obvious from studying these relatively simple trimeric compounds that there is a great deal of overlooked complexity in lignin structures. For each C_6-C_3 unit that is added to a structure, two more optical centers are added, and four times as many physical isomers become possible. What does this mean for polymeric lignin? Are crystalline regions likely in lignin, and can we expect to crystallize even small lignin fragments? It should be clear already that the number of isomer possibilities is likely to become huge for a molecule of even mild complexity.

Simple calculations show that, even for a simplified 'lignin' polymer made up solely of β-ether units, a 110-mer (DP 110 polymer) would have 10^{66} distinct isomeric physical compounds. As it turns out, 10^{66} is a good estimate for the number of atoms in the galaxy! i.e. this simple, regular, DP 110, MW 21,500, β-ether polymer has about the same number of possible physical isomers as there are atoms in our galaxy. Can you find crystalline regions in this polymer? We think not!! To take another view, suppose you wanted to obtain or find just 1 mg of crystalline 'lignin'. Under the same simplified conditions as above, if lignin was just a 10-mer, you could conceivably isolate 1 mg of any of

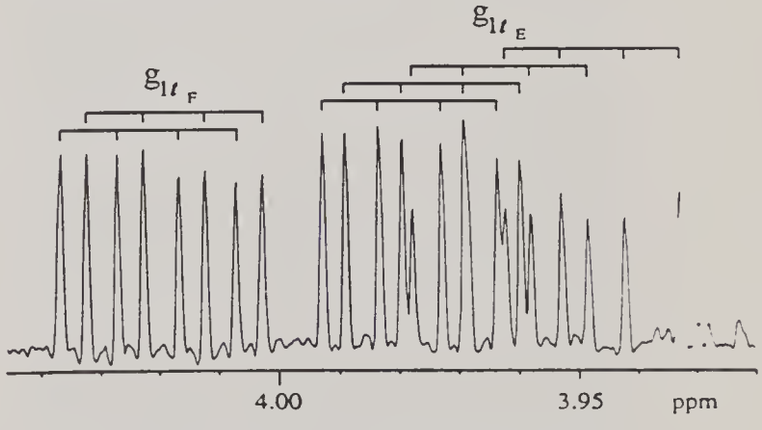


Figure 2. Expansion of a part of the γ–proton region of 3 showing dd's for each of the four isomers of the γ_E proton.

the 524,288 'lignin' decamers. If the 'lignin' is just a 47-mer however (MW ca. 9,400), you are going to need nearly twice the weight of the earth in lignin in order to isolate your 1 mg of crystalline material.

Conclusions

Trimeric lignin model compounds can be used very effectively to provide more representative compounds for determining NMR parameters appropriate to lignin structures. The spectra illustrate how any peak in the lignin spectrum will be broadened by the diversity of chemical shifts, and not solely as a result of its slow tumbling due to its molecular size. And the data from molecules such as these allow the determination of exact chemical shifts for both terminal free-phenolic end and etherified internal structures, and potentially allow for a more detailed analysis of lignin spectra by 2- or 3-dimensional spectroscopy. Finally, the difficulties associated with the purification and spectral interpretation of these relatively simple compounds document further evidence for the extraordinary isomeric complexity of lignin, that is paralleled nowhere else in the natural world.

SYNTHESIS OF METHYL 5-*O-trans*-FERULOYL-α-L-ARABINOFURANOSIDE AND ITS USE AS A SUBSTRATE TO ASSESS FERULOYL ESTERASE ACTIVITY

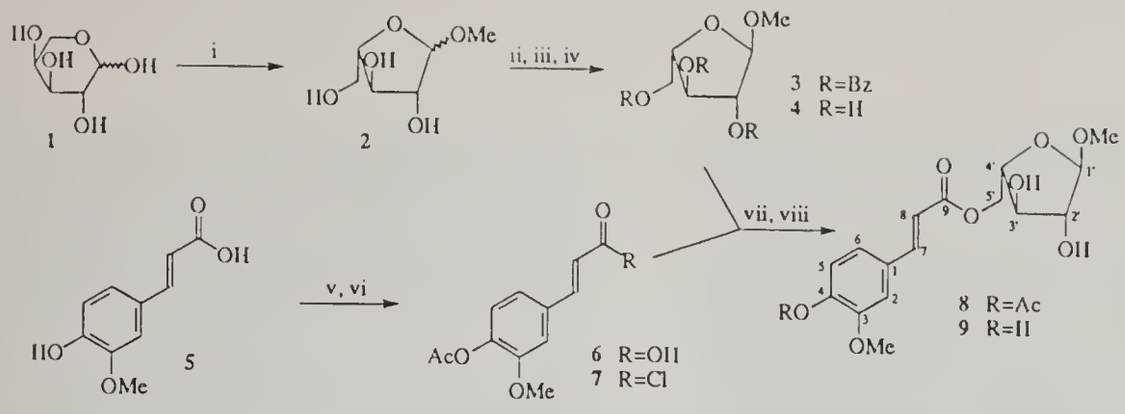
R.D. HATFIELD, R.F. HELM, and J.RALPH

Introduction

The roles of wall-bound ferulic acid have not been resolved. Ferulic acid released during saponification of cell walls is correlated with an increase in microbial and enzymic hydrolysis of wall polysaccharides, suggesting structural roles that limit polysaccharide degradation. Elucidation of the structural roles of wall-bound ferulic acid requires detailed analysis of cell-wall components. Enzymatic degradation of cell walls can generate fragments containing ferulic acid that are amenable to structural analysis. Selection of appropriate enzyme preparations requires that they be devoid of feruloyl esterase which could inadvertently remove all or part of the ferulic acid. A simple procedure was designed for the gram scale synthesis of methyl 5-*O-trans*-feruloyl-α-L-arabinofuranoside (FA-Ara) as a specific feruloyl esterase substrate. This substrate models the major feruloyl linkage to polysaccharides and provides a material with well defined chemical properties useful in the development of convenient assay procedures.

Materials and Methods

Methyl 5-O-trans-feruloyl-α-L-arabinofuranoside was synthesized from L-arabinose and ferulic acid (FA) following the procedure outlined in Fig.1. The absorption spectrum of FA-Ara and FA (0.056 μM) was recorded between 500 and 250 nm in buffer solutions (pH 5-12). Molar extinction coefficients were calculated for FA-Ara and FA for the absorption maximum at each pH. Stability of FA-Ara at high pH was determined by adding 40 μl of FA-Ara stock solution to 460 μl of 200 mM Na_2CO_3 (final pH 11.5), mixed, and the absorption spectrum recorded. Shifts in the absorption spectra were monitored at 0, 15, 30, 60, and 120 min. FA-Ara (1.38 mM) was prepared in 20 mM sodium acetate buffer (pH 5.0, 0.01% NaN_3). Reaction mixtures contained 125 μl of FA-Ara, 100 μl of enzyme mixture, and acetate buffer to a final volume of



(i) H_2SO_4/MeOH; (ii) BzCl/pyridine; (iii) selective crystallization; (iv) NaOMe; (v) Ac_2O/pyridine; (vi) $SOCl_2$; (vii) pyridine; (viii) piperidine

Figure 1. Synthetic sheme for the production of FA-Ara, 9.

200 µl. The enzyme substrate mixtures were incubated 24 h at 25 °C. A 5 µl aliquot was removed, spotted on a TLC plate, and developed in ethyl acetate:acetic acid (100:1). Substrate and the hydrolyzed FA were visualized under UV (254 nm).

Enzyme activity in preparations was determined by mixing 250 µl FA-Ara (1.38 mM) with 25 µl of enzyme solution and incubating for 20 min at 25 °C. A 40 µl subsample was removed, added to 460 µl of 200 mM Na_2CO_3, and the absorbance read at 375 nm. Direct monitoring of enzyme activity was accomplished by mixing 40 µl of FA-Ara stock solution with 435 µl of pH 7 buffer in a 1 ml cuvette. Background absorption was monitored for 4 min before 25 µl of enzyme was added. Change in absorption was recorded every min for 20 min. For HPLC analysis, enzyme-substrate mixtures were boiled for 15 min, centrifuged for 2 min, the supernatant transferred to a glass test tube, frozen in liquid N_2 and lyophilized. The resulting solid was suspended in ethyl acetate and filtered. The filtrate was evaporated to dryness and dissolved in methanol (100 µl) prior to injection. HPLC separation of FA-Ara and FA was carried out on a C_{18} column with a mobile phase of methanol:H_2O (1:1) at a flow rate of 1 ml/min. FA-Ara and FA were detected with a UV detector set at 325 nm (AUFS 0.150).

Results and Discussion

The synthesis of the methyl-5-*O*-feruloyl-α-L-arabinofuranoside (FA-Ara, 9) was straightforward as shown in the synthetic scheme (Fig.1). Analysis of UV spectra revealed that the absorption maximum of FA-Ara was distinct from that of FA over the range of solution pH values tested. The ester linkage in FA-Ara shifted the absorption maximum to higher wavelengths compared to the maximum for FA. Over the pH range used, the difference in absorption maximum varied from 17.5 to 65 nm. Because of the difference in absorption maxima, the hydrolytic conversion of FA-Ara to free FA could be monitored spectrophotometrically. A solution of 200 mM Na_2CO_3 was adequate to shift the pH to 11.5 which inactivates enzymes. Although the compound will degrade at pH 11.5, its hydrolysis is relatively slow, with losses of less than 10% over a 2 h period at 25 °C. It is therefore possible to set up several samples and read them within a relatively short time period without significant degradation. Using HPLC, it is possible to develop standard response curves for both FA-Ara and FA to quantify the amount of FA-Ara hydrolyzed and to develop automated systems for numerous samples.

Enzyme kinetics for feruloyl esterases could be determined in small volumes within the spectrophotometer cuvette by continuously monitoring the decrease in the FA-Ara maximum or the increase in the FA maximum. There is sufficient difference to allow the development of a linear response with the proper selection of time intervals and protein concentration. The activity could be monitored at any pH within the range of 5 to 12. The TLC method for monitoring activity is flexible and does not require specialized

equipment, and is sensitive for both FA or FA-Ara (1.5 nmol). TLC can be used to monitor the conversion of FA-Ara to FA which have Rf values of 0.70 (FA) and 0.52 (FA-Ara) in ethyl acetate:acetic acid. Several commercial enzyme preparations were tested with the FA-Ara compound to determine the presence of esterases capable of hydrolyzing feruloyl-arabinose-ester linkages. Of the preparations tested, half exhibited feruloyl esterase activity (Table I).

Summary

The synthesis of FA-Ara provides a specific substrate that effectively models the polysaccharide-feruloyl ester linkage that is frequently found in plant cell walls. Because FA-Ara is readily soluble in H_20, solutions can be prepared in a wide range of concentrations without first solvating in an organic solvent. Assaying hydrolytic activity against FA-Ara can be accomplished by a range of methods including TLC, UV spectrophotometry, or HPLC. There is no need for secondary derivatization to follow enzyme reactions.

Table I. Determination of feruloyl-esterase activity in commercial enzyme preparations.

Enzyme Preparation	Source	Activity
Cellulysin	Calbiochem	—
Novozym	NOVO	—
Celluclast	NOVO	—
Driselase	Sigma	—
Cellulase Type I	Sigma	++
Cellulase Type V	Sigma	—
Pectinex AR	NOVO	++
Pentosanase	NOVO	++
Pectinase	Worthington	++
Pectinase	Sigma	++

CHARACTERIZATION OF A FERULOYL ESTERASE ISOLATED FROM *HUMICOLA INSOLENS*

R.D. HATFIELD, R.F. HELM and J. RALPH

Introduction

Plant wall matrices represent stores of potential energy. Therefore, organisms that can extensively degrade plant tissues must contain a wide array of enzymes with which to attack these complex structures. Feruloyl and *p*-coumaroyl esterase activities have been identified in anaerobic and aerobic microorganisms. Such enzymes may play a critical role in the degradation and utilization of plant cell walls. Although feruloyl and *p*-coumaroyl esterases have been identified little information is available on their physical/chemical properties and substrate specificity. We are interested in the structural/functional role of ferulic acid attachment to wall polymers and the subsequent effect upon wall degradation/utilization. One objective is to obtain purified feruloyl esterases to use in cell wall structural analysis. We have partially purified and characterized a feruloyl esterase from a complex enzyme mixture produced by *Humicola insolens*.

Materials and Methods

Feruloyl esterase was purified from a mixed enzyme preparation using $(NH_4)_2SO_4$ fractionation followed by cation exchange, chromatofocusing, and size exclusion chromatography. Feruloyl esterase activity was monitored using 5-O-trans-α-L-arabinofuranoside (FA-Ara). A 40 µl aliquot of FA-Ara (1.38 mM) was added to 435 µl of 100 mM universal buffer cuvette (1 ml) and mixed. Background absorbance was monitored for 1

min at the appropriate wavelength in a spectrophotomenter. A 25 µl aliquot of enzyme sample was added to the cuvette, rapidly mixed, and change in absorbance read every 10 to 60 sec depending upon the activity. Relative activity was calculated from the rate of absorbance change. To determine the pH optimum, the esterase activity was monitored as above in universal buffers ranging in pH from 4-12. For the temperature optimum and stability trials, feruloyl esterase activity was monitored at 5, 25, 35, 45, 55, 65, or 75°C. Temperature stability was evaluated at 0, 0.5, 1, 2, 4 and 24 h in each of the temperature regimes.

Substrates tested in addition to FA-Ara included, methyl feruloylate (FA-Meth), methyl *p*-coumarate (*p*CA-Meth), methyl 3,5-methoxy-4-hydroxy-cinnamate (SA-Meth), methyl-5-O-trans-feruloyl-β-D-galactopyranoside (FA-Gal), methyl-5-O-trans-feruloyl-β-D-glucopyranoside (FA-Glc), methyl-4-O-trans-feruloyl-β-D-xylopyranoside (FA-Xyl), and methyl-5-O-trans-coumaroyl-α-L-arabinofuranoside (*p*CA-Ara).

Results and Discussion

The crude enzyme preparation from *Humacola insolens* was factionated into several major enzyme groups using ammonium sulfate precipitation and chromatographic techniques including cation exchange, chromatofocusing, and size exclusion. Enzyme activities identified included cellulase, CMCase, mixed linked β-glucanase, xylanase I, xylanase II, acetyl esterase, feruloyl esterase, and *p*-coumaroyl esterase. Only the feruloyl esterase will be discussed.

Based on size exclusion chromatography, the feruloyl esterase has a molecular size of approximately 10-12 kDaltons. This esterase maintains activity over a wide pH range with an optimum of 9. The enzyme appears to be relatively stable to a wide range of pH environments. Its temperature optimum is 55°C and loses activity if maintained at this temperature for long periods of time (20% over 24h). Activity is immediately lost at temperatures greater than 65°C.

Several substrates were evaluated to determine the hydrolytic specificity of the feruloyl esterase (Table I). The different activities shown by the ferulic acid based substrates indicate that the binding site of the enzyme must be recognizing both the phenolic and the sugar residue. Arabinose is the preferred sugar residue (alcohol of the ester linkage) being 5 times faster than the simple methyl ester. The phenolic portion also influences the hydrolysis of the ester bond. SA-Meth which has two methoxy residues on the phenyl ring (positions 3 and 5) is not hydrolyzed by the enzyme. Competitive studies with SA-Meth and FA-Ara indicate no effect on the rate of FA-Ara hydrolysis. Therefore, the lack of SA-Meth hydrolysis is due to a lack of binding to the enzyme's binding site. Based on the high hydrolytic activity against *p*CA-Ara, it would appear as if the feruloyl esterase prefers no methoxyl groups on the phenyl ring for proper binding. Competitive studies using combinations of *p*CA-Ara + FA-Ara, *p*CA-Ara + FA-Meth, and FA-Ara + FA-Meth indicate the presence of two separate enzymes. Neither FA-Ara or FA-Meth affect the hydrolysis rate of *p*CA-Ara nor does *p*CA-Ara affect the rate of FA-Ara hydrolysis. However, the combination of FA-Meth + FA-Ara does affect the rate of FA-Ara hydrolysis decreasing it by 25-30%. These results would indicate that there are two distinct esterases, one that recognizes feruloyl esters and one that recognizes *p*-coumaroyl esters.

Table I. Hydrolytic activities against several substrates. See Materials and Methods section for abbreviations.

Substrate	Activity µmloes/min/mg
FA-Ara	4.4
FA-Gal	3.1
FA-Xyl	2.5
FA-Glc	1.7
FA-Meth	0.8
pCA-Ara	13.1
SA-Meth	0

DETECTION OF FERULOYL ESTERASE IN SEVERAL PLANT SPECIES

R.D. HATFIELD, B. UICKER and R.F. HELM

Introduction

The functional role of ferulic acid has not been clearly defined. Isolation of small amounts of diferulic acid from cell wall preparations has lead to the suggestion that ferulic acid is an important cross-linking molecule between cell wall polysaccharides possibly regulating wall expansion. The discovery of ferulic acid and *p*-coumaric acid ether linked to lignin has lead to the speculation that they may act as cross-links between specific polysaccharides and lignin. Phenolic acid esterases in plant cell walls may be involved in the turnover of wall bound ferulic and diferulic acid. Controlling the spatial arrangement of ferulic acid within the matrix could regulate diferulic acid formation, and in turn, the extensibility of the wall. Esterases may also mediate the frequency and location of ferulic acid cross-links between lignin and polysaccharides. The use of non-specific substrates such as ρ-nitrophenol acetate and α-napthyl acetate may not reflect feruloyl esterase activity. Understanding the relationship between esterase activity and ferulic acid cell wall functions will require identification, isolation, and characterization of plant esterases. We have completed a small project surveying selected plant species for the presence of feruloyl esterase activity using methyl-5-*O-trans*-feruloyl-α-L-arabinofuranoside (FA-Ara).

Materials and Methods

Seedlings of corn (field and sweet), oats, wheat, alfalfa, red clover, birdsfoot trefoil, sanfoin, and mung bean were grown in total darkness for four to five days at 25°C on sterilized cheese cloth. Seedlings were separated into coleoptiles, mesocotyls, and roots (grasses) or hypocotyls and roots (legumes). Fresh tissue (1-2g) was chopped into 2 mm segments, and loaded into a 7 ml tissue grinder (Corning) with 4 ml of 50 mM KH_2PO_4 buffer (pH 7.0) containing 20 mM EDTA and 2 mM DTE. Samples were maintained on ice during grinding (10 min). Insoluble material was pelleted by centrifuging at 8500 xg for 15 min. Three, 1 ml aliquots of the supernatant were transferred to microfuge tubes (1.5 ml). One tube was capped and placed in a boiling waterbath for 15 min. After heating, the sample was centrifuged for one min and the supernatant transferred to a clean microfuge tube. FA-Ara stock solution (20 µl,1.38 mM) was added to each microfuge tube. After mixing, 5 µl was removed, spotted on TLC plates, and developed using ethyl acetate:acetic acid (100:1). Samples were allowed to incubate for 20 h at 25°C in darkness before a second sample was removed from each tube and assayed by TLC.

Oat seedlings were also analyzed by HPLC. For HPLC analysis, samples were boiled for 15 min and centrifuged for 2 min in a microfuge. The supernatant was removed from each and placed in a glass test tube, frozen in liquid N_2, and lyophilized. The resulting solid was suspended in ethyl acetate and filtered. The filtrate was evaporated to dryness and dissolved in methanol (100 µl) prior to injection. Ferulic acid and FA-Ara were separated on a C_{18} column with methanol:H_2O (1:1) as mobile phase.

Results and Discussion

FA-Ara accurately models the linkage of ferulic acid to cell wall polysaccharides. Hydrolysis of this model would be indicative of specific feruloyl esterase activity. Of the plant species surveyed, only those in the grass family exhibited substantial feruloyl esterase activity (Table I.). Alfalfa, birdsfoot trefoil, and mung bean also exhibited what may be trace amounts of activity. The presence of strong feruloyl esterase activity in the grasses may be a reflection of the higher ferulic acid con-

tent of these plants and a more prominent role in their cell walls.

To verify the TLC results, oat samples were also analyzed by HPLC (Fig. 1). Crude oat extracts contain at least one esterase that is capable of hydrolyzing the FA-Ara ester linkage. Lack of cleavage of the ester linkage by the boiled control is indicative of an enzyme mediated reaction as opposed to buffer saponification. Using HPLC it would be possible to quantify the amount of FA-Ara hydrolyzed and/or the amount of FA released. Lyophilization of the total reaction mixture after boiling to denature proteins present in the sample allows the use of small amounts of substrate. Preliminary extraction with ethyl acetate solubilizes FA and FA-Ara with a minimum of contaminating compounds (particularly ionics). Resolubilization in

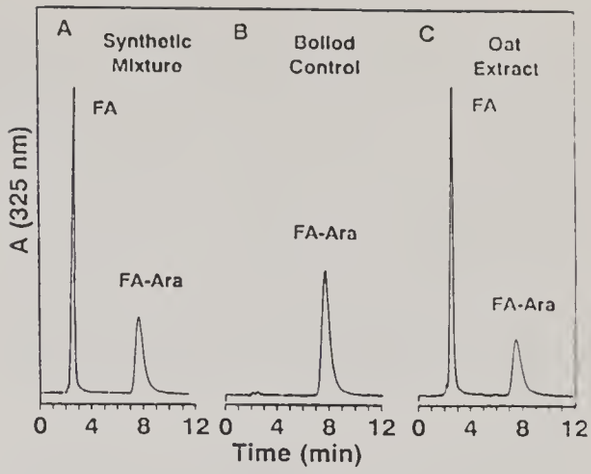


Figure 1. HPLC analysis of esterase activity in oat seedlings.

methanol allows for additional concentration of samples if necessary and dissolves the sample in a solvent similar to the mobile phase of the C_{18} column.

Table 1. Determination of feruloyl esterase activity in plants of the grass and legume families. Assays were run on cytoplasmic extracts (Cyt) and isolated cell walls (CW). Activities were evaluated by TLC, +++ = high activity, + = low activity, tr = trace, and 0 = no activity, - = not tested.

Plant species	coleoptile or hypocotyls		mesocotyl		roots	
	cyt	cw	cyt	cw	cyt	cw
corn[a]	+++	+	++	+	+++	++
oats	+++	++	++	+	+++	+
wheat	+++	+	++	-	+++	+
alfalfa	tr	tr	-	-	0	0
red clover	0	0	-	-	0	0
birdsfoot	+[b]	0	-	-	0	0
sanfoin	0	0	-	-	0	0
mung bean	tr	0	-	-	tr	

[a]all corn samples
[b]after 120h incubation

IDENTIFICATION OF A NEW CELL WALL PYROLYSIS COMPOUND

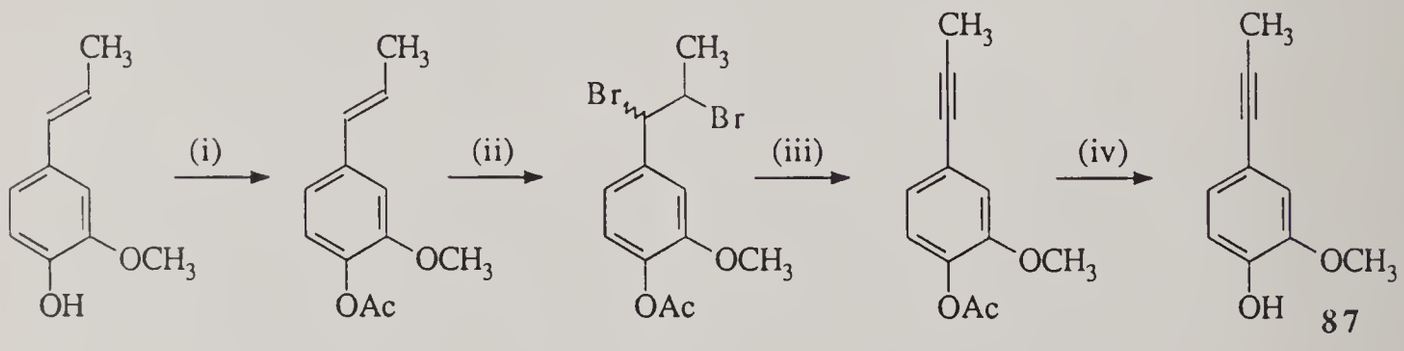
J. RALPH and R.D. HATFIELD

Introduction

In the last annual report, we reported on the use of the hybrid technique pyrolysis-GC-MS for the characterization and analysis of plant cell wall components. The preliminary identification of 130 pyrolysis components, including diagnostic carbohydrate- and protein-derived products, has finally been completed. One product that caught our attention, and that was not previously assigned but only speculated on in the literature was a product that seemed to be produced more highly from plant fractions with higher phenolic acid contents, but clearly did not arise from the phenolic acid itself. We have some speculations as to the origin of this compound (which we will leave until we gather some supporting evidence) and sought to establish its structure. This we could most easily do by synthesizing candidate compounds that were likely to give the observed mass spectrum.

Methods

Pyrolysis-GC-MS was performed as described in the 1990 Annual Report. Compound **87** (the numbering is taken from our paper on the pyrolysis of cell wall materials) was synthesized readily from *trans*-isoeugenol according to Scheme 1. Co-pyrolysis of plant materials and compound **87** were made in the standard manner by using 250 to 500 mg of plant material and spiking with 1 ml of a 1 mg/ml solution of compound **87**.



Scheme 1. Synthesis of pyrolysis product guaiacylpropyne (compound 87) from trans-isoeugenol. (i) Ac_2O/pyridine, (ii) Br_2/$CHCl_3$, (iii) DBU/toluene, (iv) NaOH/dioxane:water.

Results and Discussion

Attempts to make other isomers of guaiacylpropyne (compound **87**) including the allene isomer previously proposed, failed. The literature indicates that such allenes are likely to polymerize readily and casts doubt on the likelihood of their presence in pyrolysates anyway. However, compound **87** was shown to be the peak of interest in the following way. Pyrolysis of compound **87** left it largely intact, i.e. it volatilized under the pyrolysis conditions rather than undergoing further degradative reactions. The retention time of this peak and various peaks of interest in the cell wall pyrogram were too close to be distinguishable with certainty. However, in conjunction with the identical mass spectra of the cell wall component and compound **87**, pyrolysis of plant cell wall material spiked with authentic compound **87** confirmed its identity beyond reasonable doubt (Figure 1). The syringyl analogue of compound **87** has now also been identified in cell wall pyrolysates.

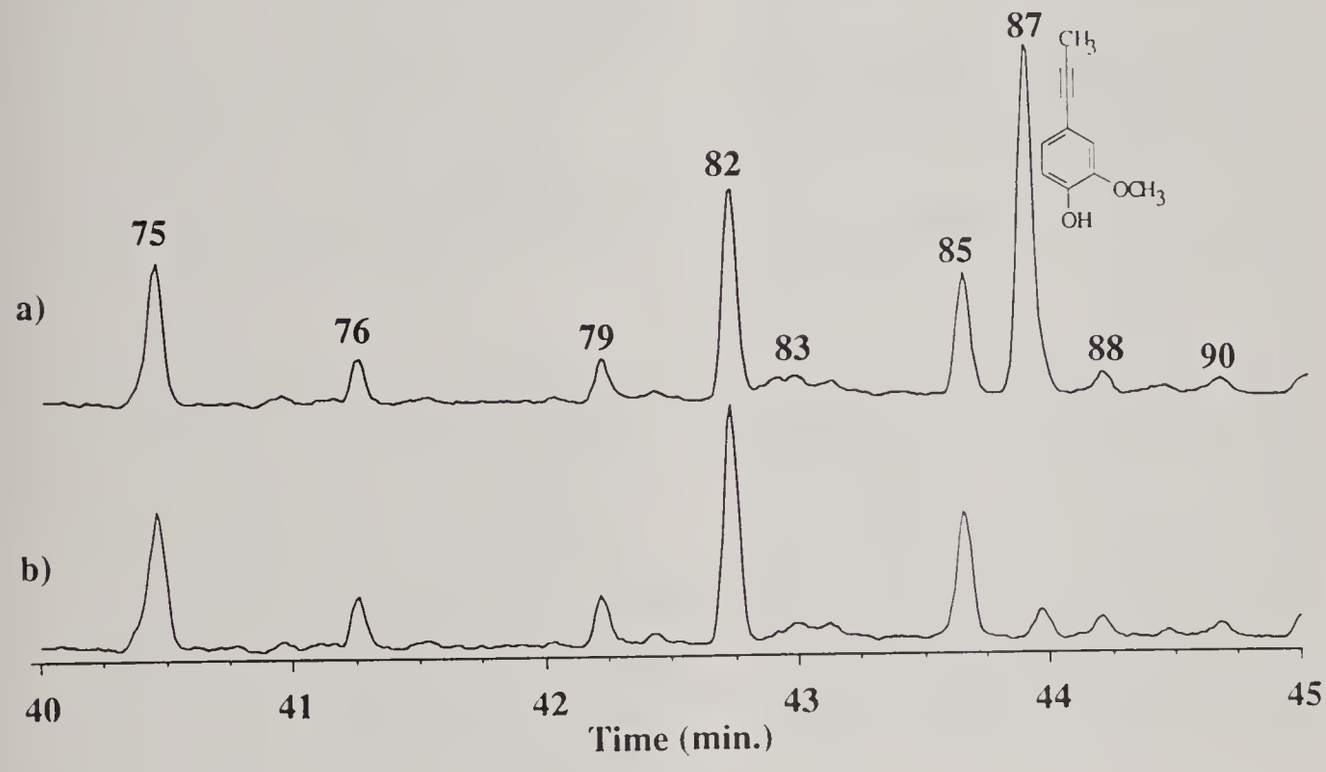


Figure 1. Partial pyrogram of alfalfa enzyme lignin, a) sample spiked with authentic guaiacylpropyne 87, b) normal sample.

REGIOSELECTIVE PROTECTION STRATEGIES FOR D-XYLOPYRANOSIDES

R.F. HELM and J. RALPH

Introduction

Arabinoxylans are an important hemicellulosic component of forages which are composed of L-arabinose and D-xylose. In their monomeric forms, these two carbohydrates are readily digestible by ruminants providing energy necessary for milk production. However, arabinoxylans are also covalently linked to phenolic acids which can in turn be attached to lignin (Figure 1). This cross-linking of hemicellulose and lignin through phenolic acids provides a three-dimensional matrix which physically restricts the rate at which the cell wall can be degraded. We are employing NMR spectroscopy to investigate the cross-linking of forage crops in an effort to determine the extent and nature of this interaction with respect to the type of forage and its maturation stage. Understanding the crosslinking phenomenon will help in developing methods to improve forage degradability and hence ruminant digestion efficiency.

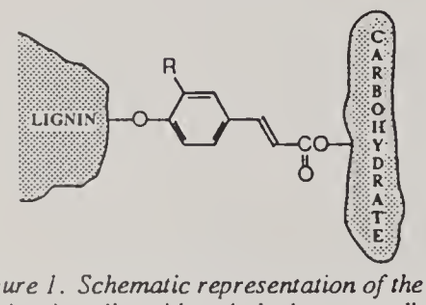


Figure 1. Schematic representation of the lignin-phenolic acid-carbohydrate crosslink.

Several appropriate model compounds were required and, as we have previously synthesized lignin-phenolic acid model compounds, the next stage was the preparation of synthetic arabinoxylans and phenolic acid-carbohydrate esters. We have developed a regioselective protection strategy for D-xylopyranosides which significantly decreases the number of steps required to provide suitable precursors for oligosaccharide production. In some cases the number of steps can be reduced from six to one with, as would be expected, a tremendous increase in yield.

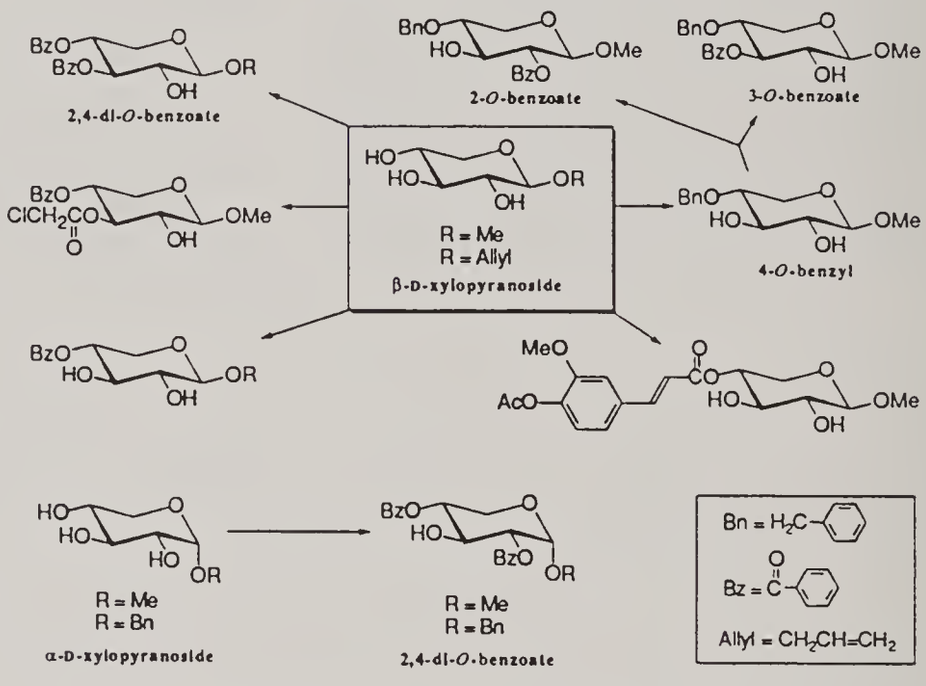


Figure 2. Use of dibutyltin oxide for the protection of D-*xylopyranosides.*

Dibutyltin Oxide-Mediated Protection

Using a modification of a recently reported technique, we were able to convert methyl β-D-xylopyranoside to the 4-*O*-benzyl derivative in high yield (Figure 2). This compound, when treated with dibutyltin oxide, formed a a mixture of stannylene acetals (Figure 3). The interesting aspect of stannylene acetals is that they "activate" one of the ring oxygens by forming a dimer. The type of dimer

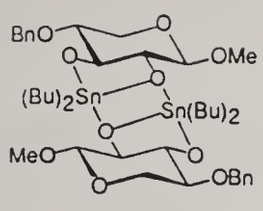


Figure 3. A stannylene acetal dimer where the 3-hydroxyl is activated.

formed depends on which is the most stable. Predicting the activated site is generally not possible, so they must be determined experimentally. When activated, the oxygen of the stannylene acetal will react with compounds such as benzoyl chloride to form a benzoate (this is termed an acylation reaction). The 4-*O*-benzyl compound formed two different stannylene acetals — in one case the 2-hydroxyl was activated, and in the other the 3-hydroxyl was. The reaction of this mixture with benzoyl chloride gave about equal amounts of the 2-*O*- and 3-*O*-benzoates which were separated in a combined yield of 85%. These two compounds have only one hydroxyl remaining, and can be used in preparing disaccharides.

The utility of dibutyltin oxide-mediated reactions for generating protected D-xylopyranosides was further demonstrated when we investigated the activation of alkyl α- and β-D-xylopyranosides. Both methyl and allyl β-D-xylopyranosides provided mono- or dibenzoates depending on the amount of benzoyl chloride added (Figure 2). The addition of 1 equivalent of benzoyl chloride gave the 4-*O*-benzoate (80% yield). A reaction with two equivalents of benzoyl chloride furnished the 3,4-di-*O*-benzoate in 90% yield. The clean conversion to mono- or di-*O*-benzoates, depending on the amount of benzoyl chloride added, allowed differentiation between the 3- and 4-positions and thus synthesis of a 4-*O*-feruloyl derivative (which depicts an ester linkage which has recently been identified in plants) as well as a 3-*O*-chloroacetyl-4-*O*-benzoyl compound. The chloroacetyl group can be removed without removing the benzoate and therefore the reaction has not only protected (in a regioselective fashion) the 3- and 4-positions, but has also differentiated between these two hydroxyls. All of this was accomplished in one step. Both benzyl and methyl α-D-xylopyranosides afforded the 2,4-di-*O*-benzoates with the addition of 2 equivalents of benzoyl chloride. There was no selectivity obtained when only one equivalent was added. However, the acylation of the 2- and 4-positions compliments the results obtained with dibutyltin oxide-mediated benzoylation of the β-xylopyranosides, and a combination of the two reactions allows substitution at any desired site.

Summary

Dibutyltin oxide-mediated acylation of D-xylopyranosides is the key step in a synthetic plan for the preparation of protected D-xylopyranosides. A variety of materials can be synthesized in high yields and in a minimum number of steps. These compounds serve as raw materials for the synthesis of arabinoxylan oligosaccharides — a topic which is the subject of another review.

THE SYNTHESIS OF ARABINOXYLAN OLIGOSACCHARIDES

R.F. HELM and J. RALPH

Introduction

It is very important to understand the chemical relationships between the major components of the plant cell wall. This information provides a sound chemical basis from which the research required to improve forage degradibility and ruminant digestion efficiency can be based. Our quest for model compounds which accurately depict the plant cell wall is the first step in utilizing NMR spectroscopy for the quantification of the relationships between lignin, phenolic acids, and carbohydrates.

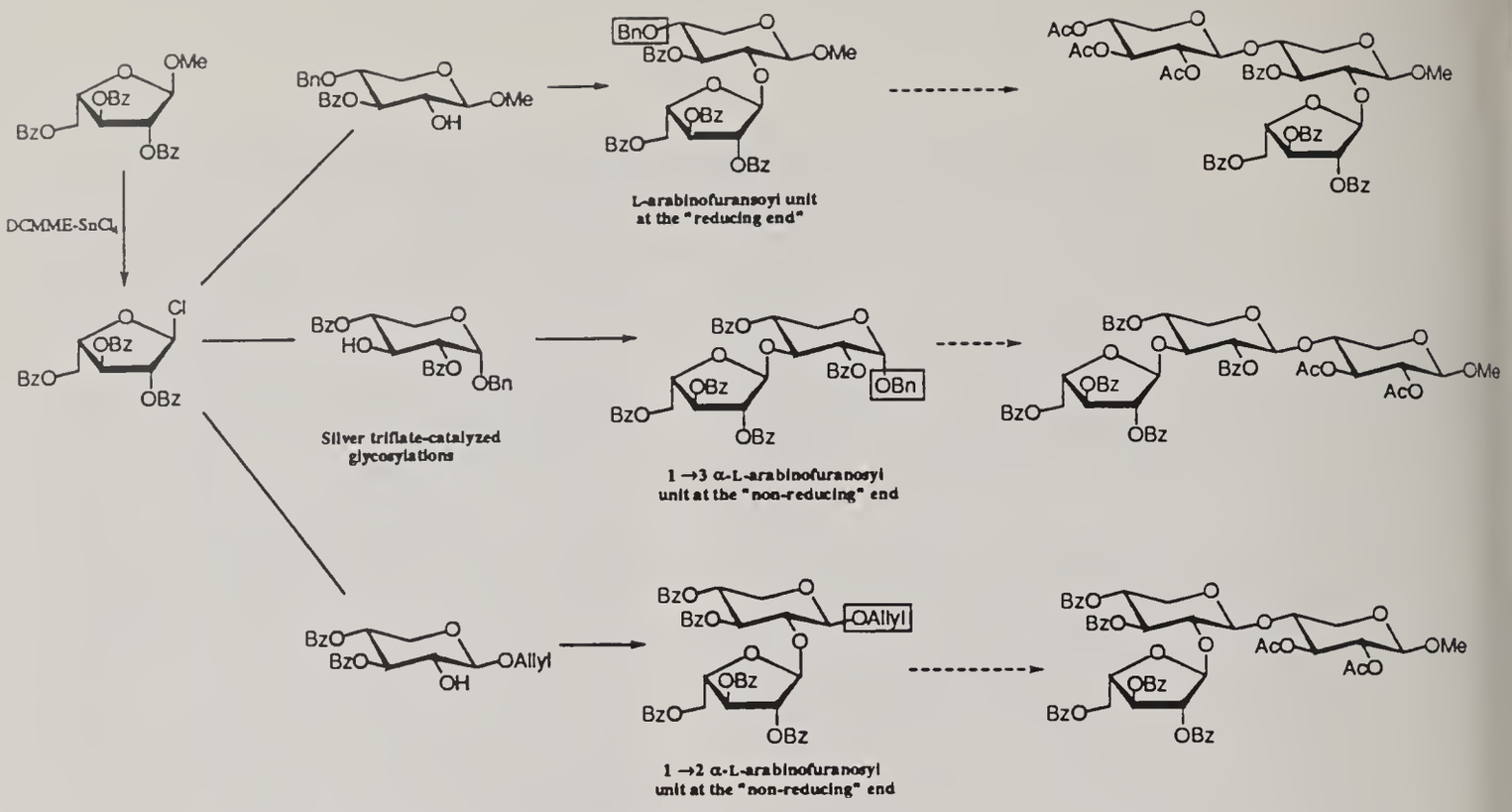


Figure 1. Regioselective approaches to arabinoxylan oligosaccharides. All of the disaccharides have been prepared. The dotted arrows indicate the nest transformations to be investigated.

The resonances of the ^{13}C atoms in the synthetic analogues will be almost exactly the same as the native material if the structures are similar. The models must be pure and fully characterized so that the assignments made with the isolated native material are unambigous. Our synthetic research has led to the development and application of new synthetic schemes which will aid not only forage and plant cell wall chemists but organic carbohydrate chemists as well. In this summary we discuss both the preparation of arabinoxylans utilizing our D-xylopyranoside protection strategy as well as the procedure for the synthesis of protected L-arabinofuranosyl chloride, a material needed for attaching L-arabinose to D-xylose in a chemically controlled manner.

Disaccharide Syntheses

A key compound necessary for arabinoxylan synthesis is an L-arabinofuranosyl halide. This material can be condensed with a D-xylopyranosidic hydroxyl affording a glycosidic bond (a glycosylation reaction). The standard technique provides an unstable material in only moderate yields. We have found that methyl α-L-arabinofuranoside tribenzoate can be converted to the corresponding chloride by the action of α, α-dichloromethyl methyl ether (DCMME) and tin chloride ($SnCl_4$) as shown in Figure 1. The reaction affords the chloride in crystalline form and in 85% yield. The material is very stable and is thus an excellent alternative to the previous technique.

For the preparation of protected β-D-xylopyranosides, the use of dibutyltin oxide proved indispensible. The formation of 2-*O*- and 3-*O*-benzoates with a benzyl group in the 4-position (Figure 1, methyl 3-*O*-benzoyl-4-*O*-benzyl β-D-xylopyranoside is shown in the upper scheme). The use of standard coupling techniques (silver triflate and collidine) afforded the α-L-linked disaccharides in surprisingly high yields (>90%). There are two possible products from a glycosylation reaction. In our cases the two possible products are the desired α-L-linkage and the unwanted β-L-bond. Conditions were developed to minimize formation of the β-L-linkge. The placement of the benzyl group at the 4-position is important because it can be removed selectively under very mild conditions. The debenzylation reaction occurs in high yield (90%) and exposes the 4-hydroxyl for eventual glycosylation with a xylopyranosyl halide. In this way the two

arabinoxylan trisaccharides with the arabinofuranosyl moiety at the reducing end can be prepared.

Preparation of the arabinoxylan disaccharides with the α-L-arabinofuranosyl moiety linked 1–3 at the non-reducing end (Figure 1, middle scheme) was accomplished by preparation of the 2,4-di-*O*-benzoate from benzyl α-D-xylopyranoside and dibutyltin oxide-mediated benzoylation. Standard glycosylation afforded the appropriate disaccharide in 89% yield. Again, the benzyl group can be removed easily, and the resulting product converted to the corresponding halide. Condensation would afford the trisaccharide. The remaining required linkage (Figure 1, bottom scheme) was accomplished by condensation of the arabinofuranosyl chloride with allyl 3,4-di-*O*-benzoyl-β-D-xylopyranoside (available from dibutyltin oxide activation in one step). Removal of the allyl group and subsequent coupling would provide the desired trisaccharide.

Summary

A protocol has been developed which allows the preparation of the four disaccharides required for higher oligosaccharide synthesis. The disaccharides were formed in high yields (90%) and were designed to be amenable to the preparation of trisaccharides. Complete NMR analysis of the synthetic compounds assures unambiguous assignments thus providing basic chemical information which will be applied directly to the analysis of the plant cell wall.

THE PREPARATION OF PHENOLIC ACID - CARBOHYDRATE ESTERS

R.F. HELM, J. RALPH and R. D. HATFIELD

Introduction

The presence of phenolic acids covalently attached to carbohydrates and lignin in forage crops decreases the efficiency with which ruminants can convert plant cell walls to utilizable substrates. Further understanding of the susceptibility of this crosslink to hydrolysis will provide the basic information needed to develop ways to improve digestion efficiency. As the ester bond between the phenolic acid and carbohydrate is the easiest to break, and ruminant microbes are known to contain esterases which may aid in hydrolyzing this bond, we have prepared several phenolic acid - carbohydrate esters with which to determine esterase substrate specificities. The synthetic procedures used are for placement of *p*–coumaric and ferulic acid moieties at the primary positions of D-glucopyranoside, D-galactopyranoside and L-arabinofuranoside. The power of modern NMR spectroscopy for structure determination is exemplified with one of the synthetic models.

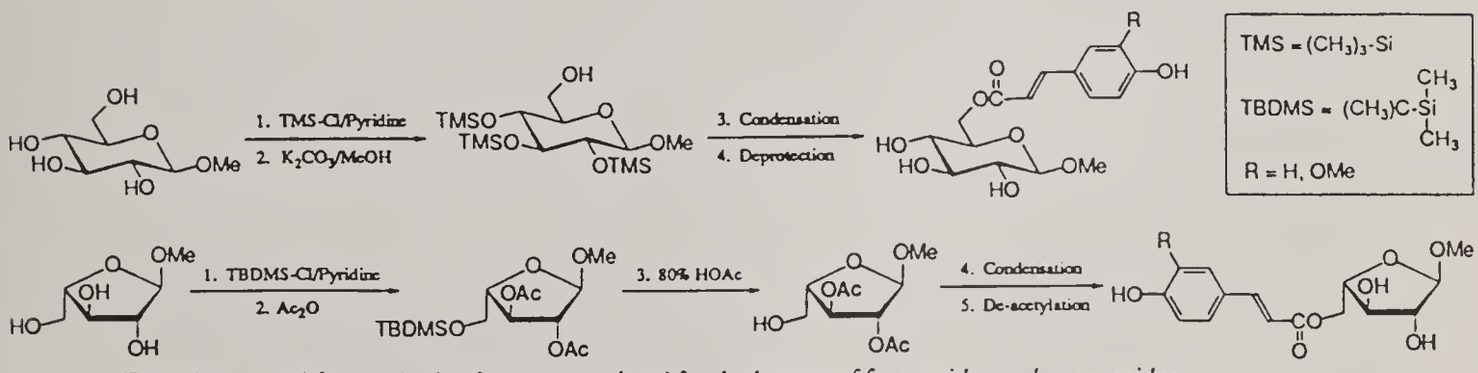


Figure 1. The scheme used for synthesis of p-coumaroyl and feruloyl esters of furanosides and pyranosides.

Discussion

The primary positions of methyl β-D-glucopyranoside and methyl β-D-galactopyranoside were readily available for condensation by complete trimethylsilylation followed by selective de-*O*-silylation of the primary position with methanolic potassium carbonate (Figure 1). Subsequent coupling with 4-acetoxy ferulic and ρ-coumaric acid chlorides gave the desired protected esters which were readily available by standard deprotection techniques. The reaction yields were quite high (80% overall for the D-galactopyranoside esters) and relatively simple. This allows easy access to phenolic acid-pyranoside esters. This procedure was not successful for methyl α-L-arabinofuranoside, and a different reaction scheme was developed. A one-pot *t*-butyldimethylsilylation/acetylation reaction provided selective protection of the primary hydroxyl (Figure 1). Cleavage of the TBDMS group with 80% aqueous acetic acid exposed the 5-hydroxyl for condensation. Subsequent deacetylation with pyrrolidine in 95% ethanol gave the *p*-coumaroyl and feruloyl esters in high yields.

Figure 2 shows the long-range ^{13}C-^{1}H correlation spectrum of methyl 5-*O*-feruloyl-α-L-arabinofuranoside. This experiment maximizes the interaction between carbons and protons which are 3 bonds away and is very useful in structural elucidation. Of particular note is the correlation between C-9 and one of the H-5' protons confirming esterification at the primary site. The carbons without attached protons were assigned from the correlations of the 3-methoxyl protons to C-3, the 4-OH to C-5 and C-3, and the H-8 and H-5 protons to C-1.

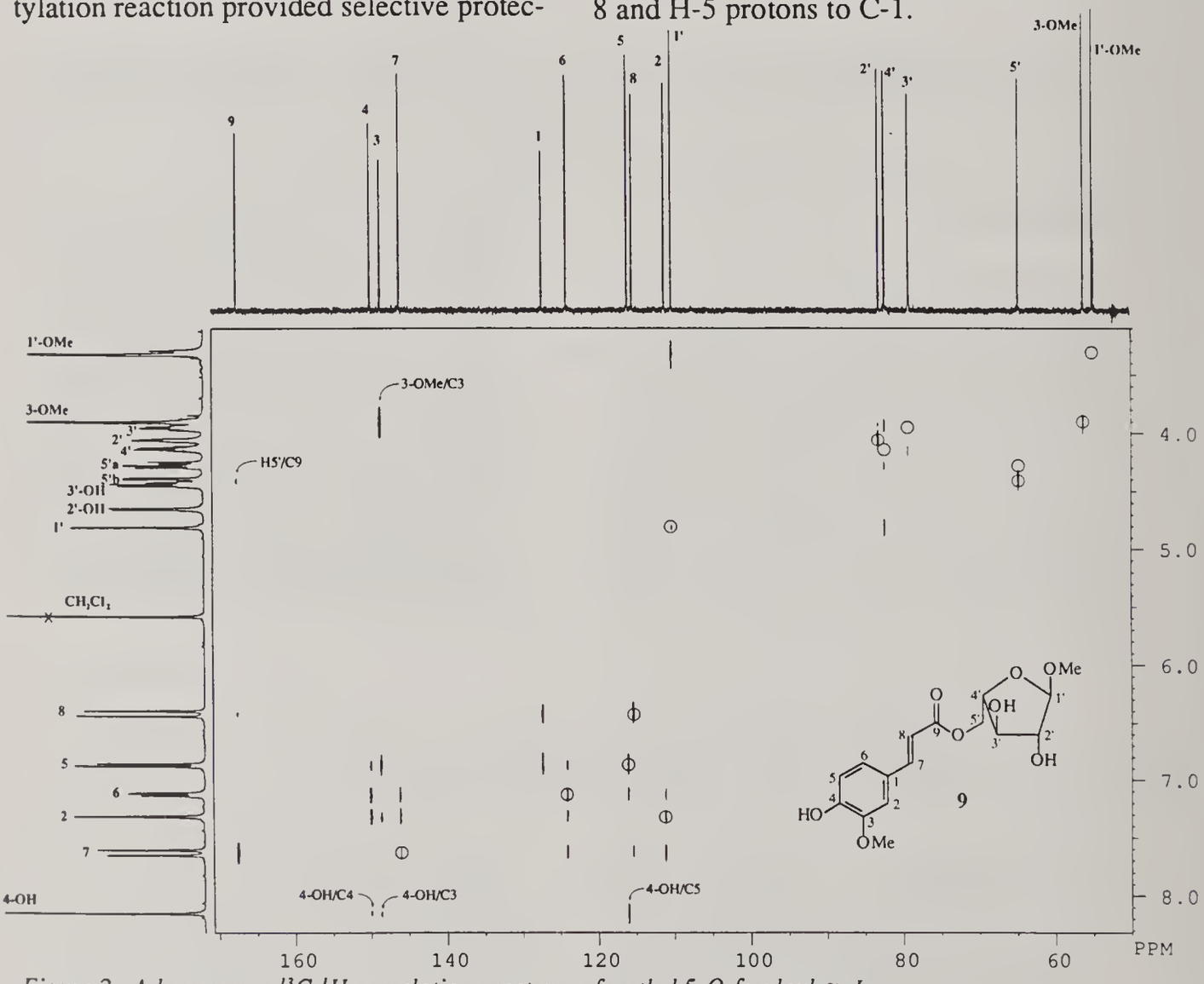


Figure 2. A long-range ^{13}C-^{1}H correlation spectrum of methyl 5-O-feruloyl-α–L-arabinofuranoside. Superimposed 1-bond correlation data is schematically indicated by open circles.

PHENYLALANINE AMMONIA LYASE ACTIVITY IN RELATION TO CHANGE IN LIGNIN CONCENTRATION AND LIGNIN DEPOSITION IN FORAGES

J.E. BIDLACK, D.R. BUXTON, R.M. SHIBLES and I.C. ANDERSON

Introduction

Breeding for decreased lignin concentration and increased digestible dry matter has been successful. These breeding efforts might be accelerated by specific selection and alteration of activity of enzymes leading to reduced lignification. Among enzymes involved in lignin biosynthesis, phenylalanine ammonia lyase (PAL) has been characterized in greatest detail and shows potential for molecular manipulation. It may be possible to improve forage quality by decreasing activity of PAL and enzymes committed to lignin synthesis and thereby decrease lignin concentration in forages. This study was conducted to determine relations between lignin concentration and content with PAL activity in stems or sheaths of maturing forages.

Materials and Methods

Six forage species, including alfalfa, birdsfoot trefoil, red clover, orchardgrass, smooth bromegrass, and switchgrass, were established in a greenhouse, and regrowth herbage was sampled at 14, 28, 42, 56, and 70 days. Sheaths of orchardgrass or stems from the other five species from the basal 10 cm of regrowth were subdivided and either frozen in liquid nitrogen for spectrophotometric PAL assays and protein determinations or dried for dry weight and sequential fiber analysis. For PAL activity and protein determination, acetone powders were prepared and stored in an ultra-low freezer at -100°C before being used. Lignin concentration and content were fitted to the Gompertz function, $y = a*\exp[(-b)*\exp(-ct)]$, where y = component measured, a = maximum value of component, b = relative growth rate as affected by t, c = estimated constant, and t = time in days. Change in concentration or content was determined from the first derivative of the Gompertz function with respect to regrowth days.

Results and Discussion

Lignin concentration and content per pot increased in all species with herbage age. First derivative plots of the Gompertz function indicated that changes in lignin concentration were often greater and decreased faster in legumes than in grasses at early days of regrowth (Fig. 1). In all species, decreasing lignin concentration changes showed a parallel relationship to decreasing PAL activity when expressed on a dry weight basis. Close relations between PAL and change in lignin concentration were consistent within species. There also was some agreement between lignin deposition and PAL content per pot (Fig. 2), but the relation was not as close as when data were expressed on concentration basis. These results suggest that there may be potential for manipulating forage lignin concentration by altering PAL activity. Altering PAL activity may also cause concomitant changes in other phenylpropanoid products because, in addition to lignin biosynthesis, PAL has a role in flavonoid, suberin, lignan, and other phenylpropanoid product biosynthesis.

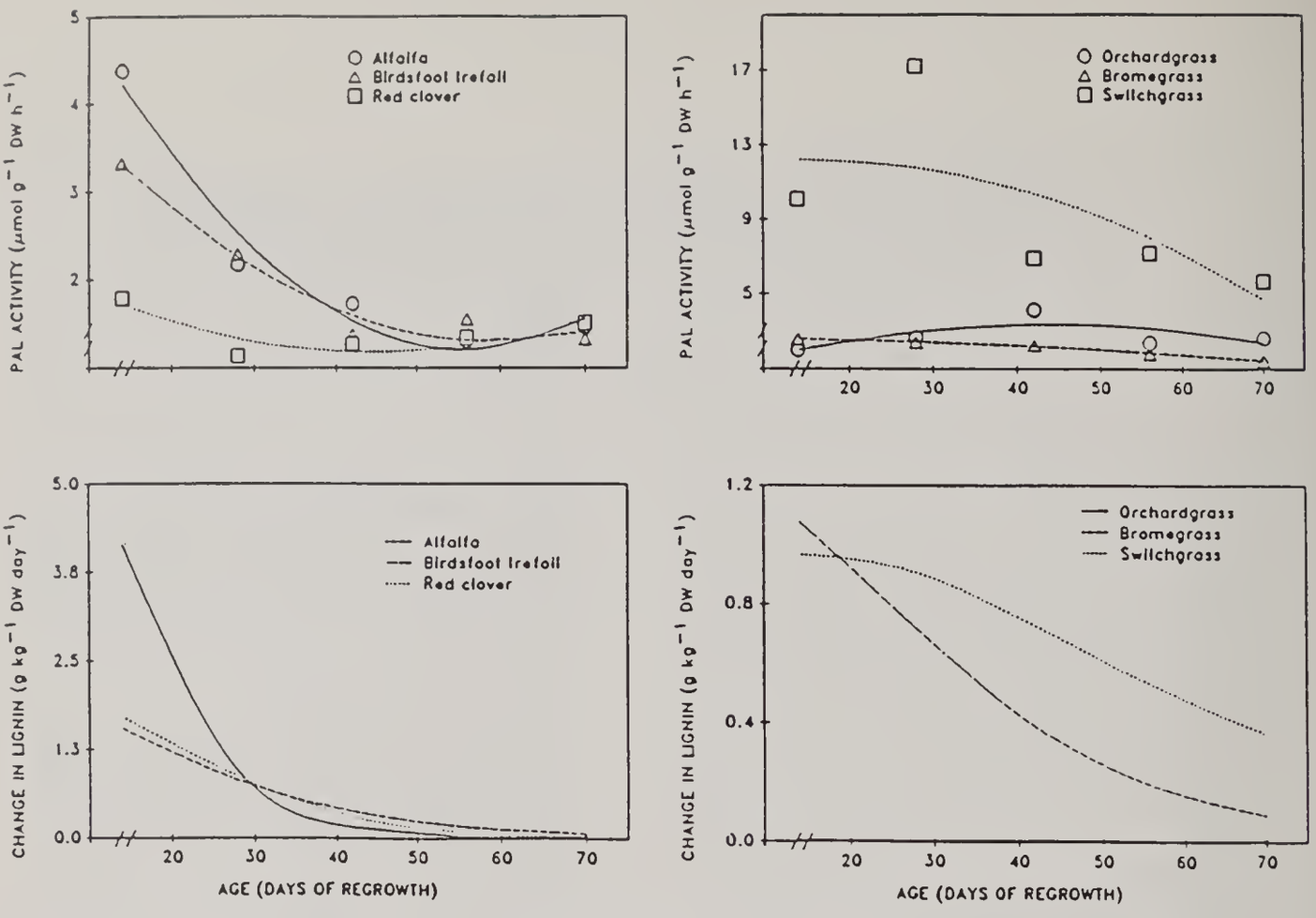


Figure 1. Phenylalanine ammonia lyase (PAL) activity and change in lignin concentration on a dry weight basis in legume and grass stems.

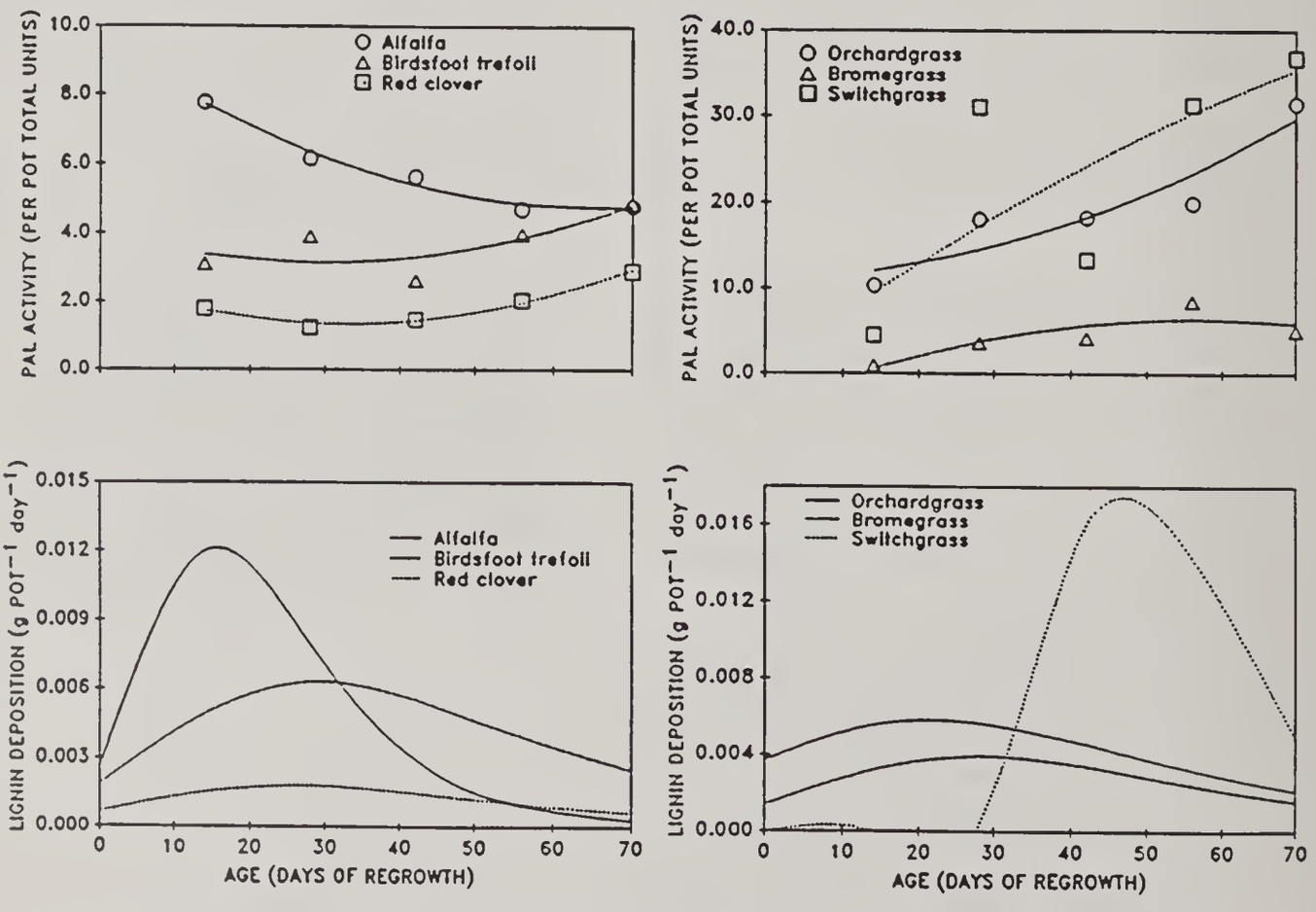


Figure 2. Phenylalanine ammonia lyase (PAL) activity per pot and lignin per pot deposition in legume and grass stems.

APPARENT INHIBITION TO DIGESTION BY LIGNIN IN NORMAL AND BROWN-MIDRIB STEMS

E.M.G. THORSTENSSON, D.R. BUXTON and J.H. CHERNEY

Introduction

Cell-wall concentration and composition have considerable impact on digestion and dry-matter intake of forages. The main cell-wall components of plants are hemicellulose, cellulose, and lignin. Hemicellulose and cellulose are at least partly catabolized by rumen microorganisms and fermented to acetic, propionic, and butyric acids, the volatile fatty acids. One factor limiting rumen degradation of these cell-wall components is their bonding to and shielding by lignin. Lignin is indigestible and can, depending on its concentration and structural composition, limit extent of digestion. Compared with their normal counterparts, the brown midrib (brm) mutants of sorghum, millet, and maize usually have reduced lignin concentrations and altered lignin compositions. Brown midrib lignin may form more intramolecular cross linkages than normal lignin which may cause each unit of brm lignin to be more inhibitory to digestion than that of normal lignin. This study was conducted to determine if in fact brm lignin is more inhibitory to digestion by rumen microorganisms than is normal lignin.

Materials and Methods

Brown-midrib and normal counterparts of sorghum brm-6, sorghum brm-18, millet KS81-1089, and maize B37bm3 grown in Indiana as well as maize B73bm1 grown in Iowa were used in this study. Stem bases from normal and bmr counterparts were harvested on 5 Sept 1989 in Indiana and on 20 Sept 1989 in Iowa from three replicates for each comparison. Samples were incubated in rumen fluid, and data were fitted with a first-order, nonlinear model to estimate concentrations of potentially digestible neutral detergent fiber (PDNDF), digestion rate of neutral detergent fiber (NDF), concentration of indigestible residue (IR), and length of lag before digestion. The NDF, acid detergent fiber (ADF), and acid detergent lignin (ADL) analyses were conducted sequentially on undigested samples. Hemicellulose concentration was calculated as the difference between NDF and ADF concentrations, and cellulose concentration was calculated as the difference between ADF and the sum of ADL-plus-ash concentrations.

Results and Discussion

The IR concentration was a function of lignin concentration (Fig. 1). The slope of the brm lines was nearly twice as large as for normal plants. Thus, each unit of brown midrib lignin, as determined by ADL, apparently was more inhibitory to digestion of NDF than normal lignin.

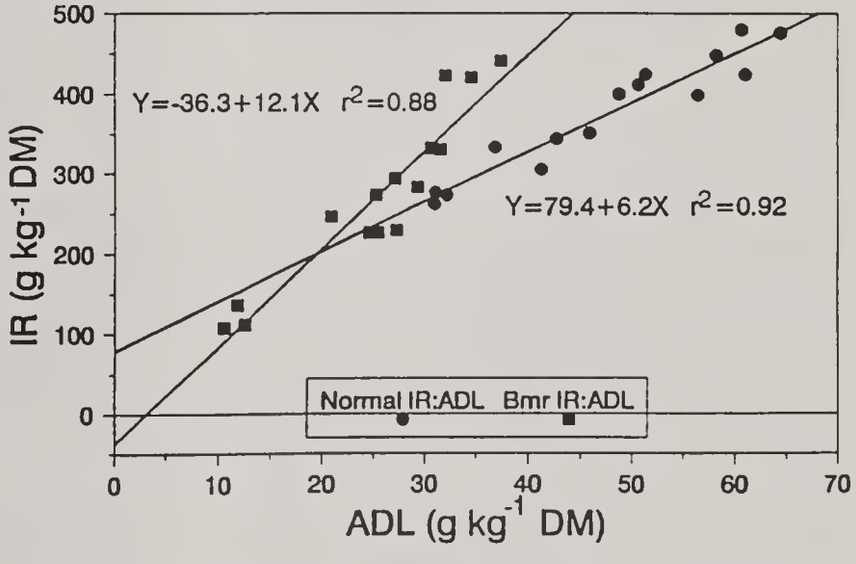


Figure 1. Indigestible residue (IR) as a function of acid detergent lignin (ADL) in normal and brown-midrib (Bmr) stems of sorghum, millet, and maize. Data are values from each replicate.

The bmr mutants were lower than normal genotypes in NDF (9%) and ADL (47%) concentrations. The PDNDF concentrations were 19% greater for bmr than for normal lines. Digestion rate of PDNDF was faster in bmr than in normal counter parts in one of the two sorghum comparisons (difference of 59%) and in the millet comparison (difference of 27%), but in neither maize comparison.

Estimation of lignin concentration by the acid detergent method yields low values because some lignin components, e.g. cinnamic acids, are hydrolyzed by the sulphuric acid. Alteration in bmr-lignin structure may enhance the hydrolization procedure and could partly explain the ADL concentration in bmr being lower than in its normal counterpart. The explanation is probably more complex than this, however, because both solubility and chemical properties differ between the lignins.

Cinnamic acids abundant in noncore lignin are believed to form cross linkages between core lignin and hemicellulose. The concentration of p-coumaric acid is usually lower, but that of ferulic acid is usually equal to or slightly higher in bmr than in normal lignin. The low cinnamic acid concentration causes a condensed lignin fraction with fewer esterbonds to hemicellulosic monosacharides. This alteration in lignin structure may be the reason that we observed a 14% greater hemicellulose-to-cellulose ration in bmr than in normal plants.

The reason for the greater apparent inhibitory effect to digestion of bmr lignin compared to normal lignin needs further investigation, but our work shows that chemical composition of lignin may be an important factor in determining its inhibitory potential.

IMPROVEMENT OF CROP RESIDUE DIGESTIBILITY WITH ALKALINE HYDROGEN PEROXIDE TREATMENT

M. AMJED, H.G. JUNG, J.D. DONKER and R.A. BLANCHETTE

Introduction

Sugarcane bagasse and the pith fraction of bagasse are by-products of sugar and paper production, respectively, from sugarcane in tropical regions of the world. Like wheat straw and other crop residues, these sugarcane residues are of low nutritional quality and do not promote high levels of ruminant animal production. Alkaline hydrogen peroxide (AHP) treatment has been shown to be very effective in increasing digestibility of wheat straw. This method is based, in part, on the biochemical mechanism for delignification employed by various fungi. In this study we assessed the potential of AHP for improving digestibility of sugarcane residues compared to wheat straw, and the changes in digestion kinetics and cell wall molecular composition were studied in detail.

Methods

Sugarcane bagasse and pith and wheat straw were suspended in a large volume of water containing 1% hydrogen peroxide. Sufficient sodium hydroxide was added to achieve a pH of 11.5. The mixture was stirred at room temperature for 24 h and maintained at pH 11.5 throughout. The insoluble residues were collected, washed with water until the effluent was neutral, and oven dried. Samples were analyzed for crude protein (CP), neutral detergent fiber (NDF), cell wall neutral sugars, Klason lignin, esterified and etherified phenolic acids, and uronic acids. Total fiber was calculated as the sum of cell wall components. *In vitro* rate and extent of NDF digestion were determined. Degradability of cell wall polysaccharide components also was measured.

Results and Discussion

Treatment of crop residues with AHP resulted in large losses of organic matter and CP (Table 1). The method will need to be modified before field use to reduce these organic matter losses which are composed of highly fermentable simple sugars and polysaccharide. Adequate protein supplementation will also be necessary. As a result of loss of solubles, both NDF and total fiber concentration of the treated residues were elevated (Table 1). The proportion of cell wall neutral sugars in the total fiber was increased by AHP treatment due to the removal of Klason lignin and phenolic acids. Uronic acid concentration remained constant. Rate of NDF digestion was increased in both sugarcane residues by AHP treatment, but not for wheat straw (Table 2).

All crop residues indicated improvement in extent of NDF digestion by AHP. Total fiber and all polysaccharide constituents were increased in degradability by AHP treatment. Alkaline hydrogen peroxide is a very effective chemical treatment. Poorer quality, highly lignified crop residues show the greatest improvement in digestibility.

Conclusion

Wheat straw was more digestible than sugarcane residues both before and after AHP treatment. However, AHP treatment improved the sugarcane residues to a greater degree than seen for wheat straw. The economic and environmental constraints on using caustic chemical agents may nullify the potential benefits of AHP.

Table 1. Organic matter loss and chemical composition of chemically delignified crop residues.

			Component[b]						
Substrate	Treatment[a]	OM loss	CP	NDF	TF	NS	KL	PA	UA
		%	- - % DM - -		% OM	- - - - - - - - - % TF - - - - - - - - -			
Sugarcane bagasse	Con	- -	2.9[c]	87.6[c]	76.8[c]	65.0[c]	29.0[c]	3.4[c]	3.1
	AHP	30.5	0.8[d]	95.5[d]	97.9[d]	78.7[d]	16.3[d]	2.0[d]	3.0
Sugarcane pith	Con	- -	3.2[c]	87.8[c]	86.5	66.0[c]	28.1[c]	2.9	3.0
	AHP	32.7	0.8[d]	94.7[d]	89.5	75.9[d]	18.7[d]	2.2	3.1
Wheat straw	Con	- -	8.2[c]	72.4[c]	76.2[c]	73.3[c]	19.1[c]	1.3[c]	6.3
	AHP	44.6	1.1[d]	91.2[d]	102.9[d]	86.0[d]	9.1[d]	0.7[d]	4.2

[a]Control (Con) Alkaline hydrogen peroxide (AHP).
[b]Crude protein (CP); neutral detergent fiber (NDF); total fiber (TF); neutral sugars (NS); Klason lignin (KL); phenolic acids (PA); uronic acids (UA).
[cd]Means in the same column, within a substrate, not sharing a superscript are different ($P<0.05$).

Table 2. *In vitro* digestion kinetics of neutral detergent fiber and degradability of cell wall constituents.

Substrate	Treatment[a]	NDF[b] Rate	Extent	TF	Cell Wall Constituents[c] NS	Glc	Xyl	Ara	UA
		/h	%	------------------	------------------	-%-	------------------	------------------	
Sugarcane bagasse	Con	.03[d]	23.0[d]	26.6[d]	44.7[d]	40.6[d]	46.2[d]	65.2[d]	47.6[d]
	AHP	.04[e]	69.0[e]	63.7[e]	80.9[d]	78.2[e]	85.1[e]	82.3[e]	74.1[e]
Sugarcane pith	Con	.03[d]	27.0[d]	35.1[d]	52.4[d]	45.6[d]	57.5[d]	63.0[d]	45.5[d]
	AHP	.06[e]	70.0[e]	60.2[d]	79.3[e]	81.0[e]	80.8[e]	81.9[e]	71.9[e]
Wheat straw	Con	.04	60.0[d]	58.2[d]	76.0[d]	72.0[d]	77.4[d]	84.1[d]	71.0[d]
	AHP	.04	94.0[e]	83.5[e]	93.6[e]	90.0[e]	97.9[e]	95.8[e]	85.8[e]

[a]Control (Con) Alkaline hydrogen peroxide (AHP).
[b]Neutral detergent fiber (NDF).
[c]Total fiber (TF); neutral sugars (NS); glucose (Glc); xylose (Xyl); arabinose (Ara); uronic acids (UA).
[de]Means in the same column, within a substrate, not sharing a superscript are different ($P<0.05$).

FUNGAL DELIGNIFICATION OF SUGARCANE RESIDUES AND WHEAT STRAW TO IMPROVE DIGESTIBILITY

M. AMJED, J.D. DONKER, H.G. JUNG and R.A. BLANCHETTE

Introduction

Crop residues resulting from the production of row crops are a major biomass resource that is of limited value to ruminant production. Since these crop residues are composed primarily of cell wall material, the high lignin content of the residues is considered to be the major limitation to their digestibility. A great deal of research has been done on the use of chemical agents for delignification of crop residues to improve their digestibility. A more benign route for improving digestibility might be through bio-delignification using aerobic lignolytic fungi. The objective of this study was to evaluate moisture levels and length of incubation as culture conditions affecting the effectiveness of a white-rot bacidomycete fungus for improving crop residue digestibility.

Methods

Wheat straw, sugarcane bagasse and the pith fraction of bagasse were the crop residues investigated. These residues were sterilized by autoclaving with sufficient water to give final moisture levels of 65, 75 and 85%. Sterile crop residues were inoculated with mycelial mats of the lignin degrading fungus *Phanerochaete chrysosporium*. The fungus was allowed to grow for 1, 2, 4 or 8 wk at 27°C and 90% relative humidity. Samples were analyzed for organic matter and Klason lignin loss; crude protein (CP) neutral detergent fiber (NDF) and 72% H_2SO_4 acid detergent lignin (ADL) content; and 48 h *in vitro* dry matter disappearance (IVDMD).

Results and Discussion

The three moisture levels tested had no effect on fungal treatment of the crop residues. Organic matter loss due to fungal metabolism of the residues was minimal the first 2 wk of incubation but became very large after more lengthy incubations (Figure 1). Removal of Klason lignin from sugarcane residues was also great, but only about 5% of the Klason lignin was removed from wheat straw (Figure 2). Fungal incubation

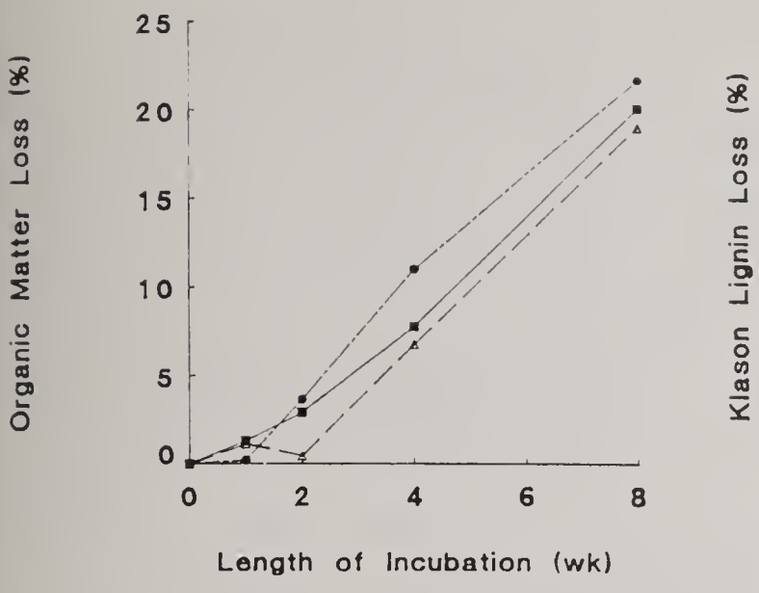


Figure 1. Organic matter loss from sugarcane bagasse (■) pith (▲) and wheat straw (●) during fungal incubation.

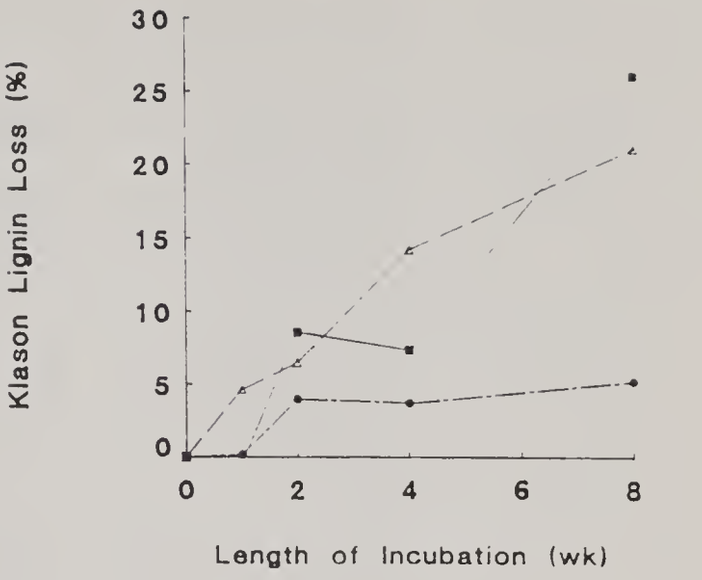


Figure 2. Klason lignin loss from sugarcane bagasse (■) pith (▲) and wheat straw (●) during fungal incubation.

resulted in increased concentration of CP in all crop residues after 8 wk, reduced NDF levels, and elevated ADL in the fiber fraction (Table 1). These data indicate conversion of cell wall polysaccharides and lignin into fungal biomass and metabolic products. The IVDMD of sugarcane residues declined during the first 2 wk of fungal treatment, but after 8 wk were significantly greater than control samples. In contrast, wheat straw IVDMD declined continually with increasing length of fungal incubation. The results can be interpreted as fungal utilization of cell solubles and easily accessible cell wall polysaccharide early during the incubation, resulting in depressed IVDMD. Longer term fungal incubation would result in removal of lignin which would increase access to previously inaccessible polysaccharide, thus increasing IVDMD. Apparently the wheat straw fiber was not sufficiently lignified to require the fungus to become highly lignolytic to expose additional polysaccharide and therefore, IVDMD never improved.

Conclusion

Phanerochaete chrysosporium is capable of improving the digestibility of very poor quality, highly lignified crop residues. The high organic matter losses encountered are a major loss of potential energy. While the percentage increases were dramatic for sugarcane residues (27 & 35%), the resulting materials were still of too poor quality for use as productive ruminant feedstuffs. Possibly longer fungal treatment could be of benefit if organic matter losses could be controlled. Finally, bio-delignification will not be practical until crop residues do not need sterilization before inoculation with the lignolytic organisms.

Table 1. Chemical composition and digestibility of crop residues incubated with *Phanerochaete chrysosporium* for various lengths of time.

Substrate	Component[a]	Control	Incubation Time (wk) 1	2	4	8
Sugarcane bagasse	CP, % DM	2.9[b]	3.5[c]	3.6[cd]	4.0[d]	4.0[d]
	NDF, % DM	88.0[b]	87.2[c]	87.0[c]	83.4[de]	80.2[e]
	ADL, % NDF	12.5[b]	14.9[b]	16.9[c]	17.1[c]	16.8[c]
	IVDMD, %	23.2[b]	20.6[c]	16.4[d]	23.7[b]	29.5[e]
Sugarcane pith	CP, % DM	2.9[b]	3.6[c]	3.6[c]	4.2[d]	4.6[e]
	NDF, % DM	89.1[b]	86.7[c]	86.9[c]	84.1[d]	78.1[d]
	ADL, % NDF	11.5[b]	16.4[c]	16.6[c]	14.9[b]	12.9[b]
	IVDMD, %	21.9[b]	22.2[b]	17.7[c]	20.7[b]	29.6[d]
Wheat straw	CP, % DM	7.5[b]	7.9[cd]	8.0[d]	7.6[bc]	9.3[e]
	NDF, % DM	75.1[b]	72.7[c]	71.0[d]	70.2[d]	70.0[dd]
	ADL, % NDF	5.4[b]	6.6[cd]	5.9[bc]	6.3[c]	6.9[d]
	IVDMD, %	58.3[b]	58.2[b]	55.4[c]	52.7[d]	46.7[e]

[a]Crude protein (CP); neutral detergent fiber (NDF); acid detergent lignin (ADL); *in vitro* dry matter disappearance (IVDMD).
[bcde]Means in the same row not sharing a superscript are different ($P<0.05$).

STABILITY OF QUALITY TRAITS IN STEMS OF ALFALFA

R.R. SMITH and C.S. ENDRES

Introduction

Alfalfa plant parts vary in nutritive value. Leaves are highly digestible as are young stem tips, while the digestibility of stems is relatively low due to their higher cell wall content and greater degree of lignification. Leaf digestibility remains high with advancing maturity; however, stem digestibility, particularly of the lower stem segments, declines rapidly with ontogeny and is lower in quality than leaves at all stages of maturity. Total herbage based selection has resulted in greater leaf-to-stem ratios whereas changes in stem concentration and composition have been small and somewhat unpredictable. Little information is available on the degree of genetic variability for quality traits in legume stems and the influence of stage of growth, regrowth period, and/or environment on this variability. Documenting the stability of quality traits in stem tissue over various growth periods involving environmental and maturity differences would aid breeders in choosing a selection criteria for improvement of alfalfa stem quality. The objective of this study was to determine the stability in performance of alfalfa genotypes for stem quality traits as influenced by the environment, the stage of growth, and the growth period.

Materials and Methods

Six propagules of seventeen alfalfa genotypes ranging from 380 to 550 g kg^{-1} for stem in vitro dry matter digestibility (IVDMD) were transplanted in a randomized complete block design with three replications at Arlington and Prairie du Sac, WI in 1987. In 1988, the lower stem tissue of two propagules per genotype per replication was harvested at three growth stages (bud, 50% bloom, and green seed stage) for each of two growth periods (harvest I and II) at each location. NIRS technology was used to predict IVDMD, neutral and acid detergent fiber (NDF and ADF) and acid detergent lignin (ADL) after adjusting, if necessary, for maturity differences between genotypes.

Results and Discussion

Significant differences among genotypes for IVDMD, NDF, ADF, and ADL were detected at each stage of growth, growth period, and location. At both locations the interaction between genotype and stage of growth was significant for all quality traits at the first growth period (harvest I) but not the second (harvest II), indicating an unstable genotypic response between stages at the first harvest but not the second. When combined over locations, the genotype by location interaction was significant for all quality traits, again indicating a lack of genotypic stability in performance (Table 1). These significant interactions should suggest that the phenotypic correlation of genotype performance for these quality traits between stages of growth, growth periods and locations would, in fact be quite low. This was not the case (Table 2). Closer examination of the ranking of the genotypes suggests that the significant interactions are the result of the magnitude of differences between genotypes and not due to their relative ranks at the different harvests. Of the four quality traits, ADL was the most stable and predictable trait between stages and locations, especially at the first harvest period (9 of 11 r values > 0.7) (Table 2). These results suggest that selection for alfalfa stem IVDMD or ADL would be most effective during the first growth phase of the season; however, it would be appropriate to evaluate material at more than one growth stage and location. This would be especially applicable if the objective was to improve stem quality at the bud stage of growth.

Table 1. Significance of mean squares combined over stages of growth (Sta), growth periods (Har), and locations (Loc) for IVDMD, NDF, ADF, and ADL in alfalfa stems.

Source	df	IVDMD	NDF	ADF	ADL
Genotype (Gen)	16	**	**	**	**
Gen x Loc	16	**	**	**	**
Gen x Sta	32	*			
Gen x Sta x Loc	32			**	
Har x Sta	2	*			
Har x Gen	16		**	**	**
Har x Gen x Loc	16	**			

*,**Significantly different at 5 and 1% level, respectively.

Table 2. Phenotypic correlation coefficients for alfalfa genotypic performance between growth stages (S), growth periods (H), and locations (L) for stem IVDMD, NDF, ADF, and NDF.

Pairs of Growth periods		Trait			
		IVDMD	NDF	ADF	ADL
AH1S1 vs	AH1S2*	0.44	0.62**	0.68**	0.89**
	AH1S3	0.30	0.20	0.20	0.80**
	AH2S1	0.10	0.10	0.13	0.83**
	AH2S2	0.36	0.43	0.63**	0.69**
	AH2S3	0.45	0.43	0.62**	0.71**
	PH1S1	0.72**	0.72**	0.73**	0.03
	PH1S2	0.67**	0.67**	0.60*	0.84**
	PH1S3	0.13	0.30	0.32	0.74**
	PH2S1	0.49*	0.67**	0.65**	0.70**
	PH2S2	0.55*	0.44	0.12	0.76**
	PH2S3	0.59*	0.47	0.53*	0.27

*Code: AH1S1 = Arlington - Harvest 1 - Stage 1, etc.
*,**Significantly different at 5 and 1% level, respectively.

ECONOMICS OF SWATH MANIPULATION DURING FIELD CURING OF ALFALFA

C.A. ROTZ and P. SAVOIE

Introduction

Hay swath manipulation can improve the rate and uniformity of drying by aerating the bottom, wetter layers of the curing swath. Disadvantages of the process are the mechanical losses and increased costs of the additional machine operation. Two types of swath manipulation exist. Tedders lift, fluff and spread the swath for more rapid drying, and swath inverters lift and turn the swath over to expose the wetter material on the bottom of the swath. The economic value of swath manipulation is difficult to determine. A long-term analysis is needed to balance the costs and benefits. DAFOSYM provides an excellent tool for performing this type of analysis. DAFOSYM integrates the effect of enhanced drying on forage quality, ration formulation for a dairy herd and the total cost of harvest, storage and feeding.

Objectives of this work were: (1) To integrate models of tedding and swath inversion and their effects on alfalfa drying rate and loss into DAFOSYM, (2) To analyze the economics of these treatments on a representative dairy farm for various strategies with regard to the timing of swath manipulation and (3) To determine the sensitivity of the economic analysis to climatic region, farm size, machine size and other machine and treatment parameters.

Materials and Methods

To model the tedding and swath inversion operations in DAFOSYM, parameters and functions were added to describe maximum field speed, field efficiency, operating width, mass, initial cost and power requirement for each machine. Functions were also required to describe drying rate and loss. For tedding, a 30% increase in drying rate was assumed on the day the treatment was applied. Tedding loss was modeled as a function of the crop's moisture content at the time of tedding. Swath inversion was modeled to increase drying rate 15% on the day the treatment was applied, with little effect on following days. A loss model for swath inversion predicted a loss of 0.7 to 1.5% of the crop dry matter or about 20% of the value predicted for tedding loss.

To analyze the benefits of swath manipulation in field-curing alfalfa, the process was simulated on a representative dairy farm of 60 cows with 30 ha (74 acres) planted in alfalfa and 30 ha planted in corn. A three-cutting alfalfa harvest system was used in which all alfalfa was baled in small, rectangular bales. The analysis was performed for 26 years of East Lansing, Michigan weather and 10 years of weather from Québec City, Canada. A variety of alternative strategies for swath manipulation were evaluated to determine their economic benefit. These included six alternative systems that used combinations of tedding and raking and six systems that used swath inversion (Table 1).

Results and Discussion

Tedding alfalfa once soon after mowing reduced the average field curing time by 13 h in first cutting and 6 h in subsequent cuttings (Table 1). Tedding more than once provided little additional benefit through faster drying. Applying inversion once either on the second day of curing or after rainfall reduced the average field time by 5 to 6 h in third cutting and 1 to 4 h in other cuttings. Mechanical losses caused by tedding were greater than the

average rain-induced loss avoided by using the process. With little improvement in the quantity and quality of hay produced, additional machinery and labor costs of tedding decreased farm income. Swath inversion caused less loss, but costs were higher giving a similar range in the loss of farm income. Simulation of the systems on the same farm in Québec Canada with two cuttings of alfalfa gave similar results. On the East Lansing farm, the most economical system was to dry hay in narrow swaths with no manipulation, but with Québec weather the most economical system dried hay in a wide swath with raking prior to baling. The economic value of swath manipulation treatments was not highly dependent upon any of the major model parameters assumed in the analysis.

Table 1. Effects of swath manipulation treatments on field-curing time, hay production and costs for a dairy farm of 30 ha of alfalfa, 30 ha of corn and 60 milking animals located in East Lansing, Michigan.

	Field curing time			Hay produced		Hay quality		Production[1]	Feed[2]
System*	Cut 1 (days)	Cut 2 (days)	Cut 3 (days)	Hi-qual. (t DM)	Low-qual. (t DM)	CP (%)	NDF (%)	cost ($/t DM)	cost (%)
A	4.5	3.8	5.2	118	152	20.4	43.4	76	40.2
B	6.0	4.4	5.7	115	149	20.4	44.3	73	39.7
C	4.3	3.7	5.0	121	149	20.4	43.3	80	40.8
D	3.9	3.5	5.0	124	138	20.3	42.9	83	41.5
E	4.1	3.6	5.0	109	145	20.2	43.4	85	41.7
F	3.8	3.5	4.9	113	138	20.0	43.3	88	42.3
G	5.9	4.4	5.4	113	148	20.4	44.1	80	40.9
H	4.5	3.8	5.2	117	154	20.4	43.4	76	40.2
I	4.3	3.8	5.0	121	146	20.4	43.2	83	41.2
J	4.4	3.8	5.0	121	149	20.4	43.3	78	40.5
K	4.3	3.8	5.0	120	148	20.4	43.2	81	40.9
L	4.2	3.7	5.0	124	144	20.3	43.0	84	41.6

*(A) wide swath, raked prior to baling; (B) narrow swath, not raked; (C) wide swath, tedded following rain, raked prior to baling; (D) wide swath, tedded soon after mowing and following rain, raked prior to baling; (E) wide swath, tedded day following mowing and following rain, raked prior to baling; (F) wide swath, tedded soon after mowing, day following mowing and following rain, raked prior to baling; (G) narrow swath, inverted day following mowing; (H) wide swath, inverted to narrow swath prior to baling; (I) wide swath, inverted day following mowing and following rain, raked prior to baling; (J) wide swath, inverted following rain, inverted to narrow swath prior to baling; (K) wide swath, inverted day following mowing and following rain, inverted to narrow swath prior to baling; (L) wide swath, inverted twice during curing and following rain, inverted to narrow swath prior to baling.

[1]Includes all costs for production, harvest and storage of alfalfa hay on the representative dairy farm.

[2]Includes all costs for production, harvest, storage and feeding of alfalfa, corn silage and corn on the dairy farm expressed as a percent of milk income.

QUICK-DRYING FORAGE MATS

R.G. KOEGEL, T.J. KRAUS, R.J. STRAUB, and K.J. SHINNERS

Introduction

Mats made from alfalfa macerated at the time of mowing and placed on the stubble have been shown in earlier research to dry to a moisture content suitable for baling in less than 6 hours under favorable conditions. Furthermore, alfalfa so harvested proved to have more rapid and extensive dry matter digestibility.(generally 10-15% increase) than conventionally harvested material.

Efforts during the past year have concentrated on (1) evaluation of an alternative maceration process which appears to have the potential of simplifying equipment, making it less vulnerable to foreign objects, and reducing power requirements and (2) production of mat-harvested and control hay and silage for feeding trials with fistulated cattle with the goals of learning more about the utilization of additional energy derived from macerated forage (milk production vs. body weight increase), and the level of "by-pass" protein relative to conventional forage.

Materials and Methods

An alternative mowing and macerating unit (Fig. 1) was developed and evaluated. This unit consists of two crushing rolls which flatten the alfalfa stems causing longitudinal cracks. The cracked stems are then impacted by ridges on a third rotor which causes fiberization of the stems and disintegration or severe bruising of leaves and softer parts. Mowing is accomplished by a flail mower. This unit, when combined with a suitable press, would constitute an alternative mat machine with a greatly reduced number of moving parts.

A pre-existing forage mat-making machine and a mat-harvesting unit were used to produce both dry hay and silage for comparison with conventionally harvested forage from the same field in feeding trials. As in the preceding year, both mat- and conventionally-harvested silage was stored in 6 mil plastic bags intended for round bales. Mats and conventional hay were harvested as rectangular bales.

Results and Discussion

Evaluations of degree of maceration were carried out using a Surface Area Index (SAI) which is a standardized procedure to measure rate of moisture absorption in previously oven-dried material. Variables assessed included field speed, crushing roll speed and force, and impact rotor speed. General conclusions were that satisfactory maceration could be achieved at speeds of 5 mph and above with energy requirements ranging from 0.7-1.0 kWh/t (wet basis) excluding the flail mower which is well below the requirements of the pre-existing macerator. The relative simplicity of the machine adds to its appeal.

The results of feeding trials involving mat-harvested forage are reported elsewhere in this publication by Mertens and Hintz.

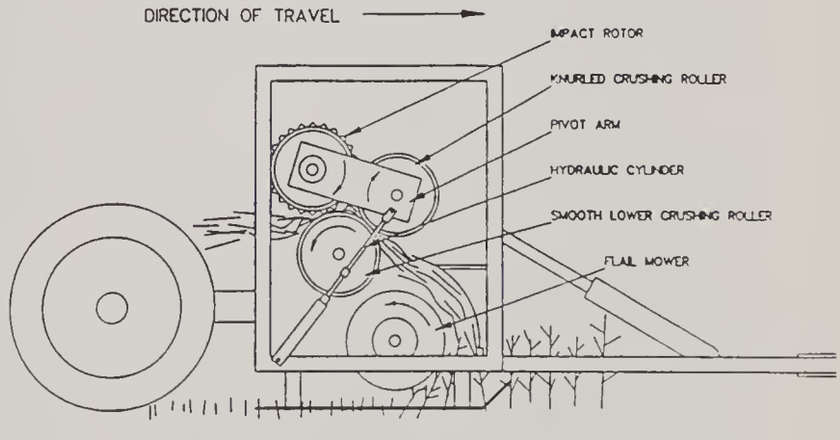


Figure 1. Schematic of Crushing-Impact Macerator.

ENERGY REDUCTION IN FORAGE HARVESTING

R.G. KOEGEL, K.J. SHINNERS, and R.J. STRAUB

Introduction

The power requirement of forage harvesting is frequently a limiting factor which determines the rate at which harvesting can be accomplished. Since timeliness of harvest reduces risk of weather damage, a lower power requirement can lead to improved forage. In addition, use of a smaller power unit reduces both capital and operating costs.

Approximately 40% of power required by a forage harvester is used for the blower which functions at approximately 5% efficiency. The method of forage conveying, therefore, appears to merit investigation relative to possible improvements.

Materials and Methods

Two approaches were investigated: (1) A forage cutterhead was inverted, so the kinetic energy imparted to the forage by the knives, rather than a blower, was used to convey the chopped forage to the trailed wagon; and (2) small quantities of water (15 gal/hr) were injected into the blower housing to act as a lubricant between the forage and the band.

Results and Discussion

The inverted cut-and-throw cutterhead forage harvester typically operated with a specific energy requirement which was 30% less than that of a conventional cut-and-blow forage harvester (Table 1). Its ability to convey to the rear of a forage wagon was generally considered adequate. It is believed possible that with blade modification, performance of the inverted cutterhead could be further enhanced.

The cut-and-blow forage harvester with injection of water lubrication (15 gal/hr) into the blower had a specific energy requirement 14% lower, under gumming conditions, than without lubrication (Table 2). This is equivalent to a 35% reduction in the blower. When gumming conditions are not present, the benefits of lubrication are greatly reduced.

Table 1. Energy requirements of upward cutting cut and throw, conventional cut and throw and conventional cut and blow harvesters.

Machine Configuration	Moisture Content (% wb)	Feed Rate (t/h)	Adjusted Specific Energy (kWh/t)	Mean Length of Cut (mm)	Number of Tests
Cut and Blow	63.4	13.5	1.98	10.9	18
Conventional Cut and Throw	64.0	13.8	1.80	11.4	18
Upward Cutting Cut and Throw	64.0	13.2	1.25	12.0	18
LSD	4.7	4.4	0.35	0.6	

Table 2. Energy requirements with lubricants at common flow rates with gumming present in forage harvester blower.

Lubricant Rate	Moisture Content (% wb)	Moisture Feed Rate (t/h)	Adjusted Specific Energy (kWh/t)	Obser.
None (0 L/h)	50.8	17.9	2.10	6
Water Only (57 L/h)	50.2	19.1	1.78	6
Water + Wetting Agent (57 L/h)	47.3	16.7	1.74	6
LSD		2.8	0.23	

INFLUENCE OF RAIN AND CROP CHARACTERISTICS ON ALFALFA DAMAGE

C. A. ROTZ, R. J. DAVIS and S. M. ABRAMS

Introduction

A great frustration for a hay grower is to have a high quality alfalfa crop damaged by rain during field curing. It has long been recognized that rain on a wilted forage crop reduces yield. Rain damage also reduces the nutritive value of alfalfa, and this loss may have greater value to the animal consuming the forage than the loss of yield. In order to develop more accurate models to predict the effects of rain damage to alfalfa, more information is needed on how crop and rainfall characteristics affect damage. This study was undertaken to determine basic information on the rain and crop characteristics that affect alfalfa damage and the interaction of these characteristics.

Materials and Methods

Artificial rainfall was created with a spray system in an enclosed structure. The structure, built of steel pipe covered with vinyl tarp, was divided into three bays with a nozzle located over the center of each bay. Water flow and pressure were set for each nozzle to enable simultaneous application of three rainfall treatments. Rain damage was measured on samples held in screen trays measuring 0.5 m square. Alfalfa samples were gathered in the trays and field dried to the appropriate moisture content. For exposure to rain, the trays were set on the field surface inside the structure where they remained on the wet soil for the duration of the rainfall. Following the rain, the trays were removed from the structure and placed on the alfalfa stubble for redrying.

Five experiments were conducted to evaluate different main effects and interactions. Main effects included amount, rate and duration of rainfall, swath density, conditioning treatment, crop moisture content at rainfall and the time of day rain occurred. Each experiment was repeated in three trials, one for each of three cuttings. Experiment 1 was used to measure the effects of swath density and duration of rainfall. Alfalfa samples of 400, 800, 1200 and 2000 g DM/m^2 were exposed to

rainfall durations of 0, 1, 3.5 and 7 h. Experiment 2 measured the effects of the rain amount, swath density, and the crop moisture content at the beginning of rainfall. Samples of 400 and 1200 g DM/ m^2 were field dried to 65, 40 and 25% moisture content and exposed to 0, 5, 18 and 52 mm of rainfall.

A new process of maceration and mat drying of alfalfa offers very fast drying, but heavy processing of the crop leaves it more susceptible to rain damage. Experiment 3 evaluated the effects and interactions between the amount of rain and the conditioning the crop received. Three treatments were compared: mechanical conditioning, mechanical conditioning plus chemical conditioning, and maceration with mat drying. Alfalfa samples from each conditioning treatment were placed in trays at a density of 800 g DM/ m^2. Rain was applied after all samples of a given treatment had field dried to 40% moisture. A rate of 18 mm/h was used for a total of 0, 18 and 62 mm of rainfall.

Experiment 4 was done to determine if the time of day rain occurred had an effect on loss. The rationale was that hay receiving rain late in the day would lie in the field wet all night. Wet alfalfa would encourage microbial growth and thus, increase loss. Mowing of the alfalfa was timed to provide alfalfa at about 40% moisture at the time of day the rain was to occur. Half of the samples were exposed to rain at 11:00 a.m. and the others were exposed at 5:30 p.m. Rainfall amounts of 0, 6 and 25 mm were used with a rainfall duration of 1 h.

Experiment 5 was designed to measure the effects and interactions between the rate or intensity of rainfall and crop moisture content at the time of rain. Alfalfa samples of 400 and 1200 g DM/m^2 were dried to either 65 or 40% moisture before rain occurred. Rainfall rates of 0, 4 and 50 mm/h were applied over 0, 6 and 0.5 h, respectively.

Measurements of rain damage to the alfalfa were: total DM loss, leaf loss and the change in nutrient concentration. Leaf loss was measured as caused by rain and mechanical handling. Rain induced loss was all material remaining in the tray when the field-dried alfalfa was gently lifted from the tray at the completion of a trial. Loss induced by mechanical handling was measured by lifting the alfalfa from the tray, inverting the material and dropping it about 0.6 m onto a hard surface. Material dislodged from the matted alfalfa was measured as loss. Measures of nutritive value were crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent insoluble protein (ADIP) and in vitro dry matter digestibility (IVDMD).

Results and Discussion

The amount, rate and duration of rainfall were closely related and each had an effect on rain damage. Total DM loss in alfalfa was 0.1% DM/mm of rainfall when the rate of rainfall was held at 18 mm/h. With the amount of rainfall held at 18 mm, the total DM loss was 1%/h of rain duration. Loss was not affected by the time of day rain occurred. Nutrient concentration in the harvested crop was greatly affected by rain damage. Dry matter leached from the plant (total loss minus leaf loss) was highly soluble material, primarily nonstructural carbohydrate and protein. The increase in fiber (NDF and ADF) concentrations and the loss of IVDMD were directly related to the loss of DM. Protein (or plant nitrogen) leached from the crop by rain was not lost as rapidly as other DM, so CP increased slightly in concentration following rain. The protein lost was soluble protein. The concentration of ADIP increased, and the increase was often greater than that explained by elution of soluble protein.

The measured characteristics of the alfalfa crop had less effect on rain damage than the rainfall characteristics. Swath density of the alfalfa did not have a consistent effect on the total DM loss and nutrient change in rain damaged alfalfa. Leaf loss from heavy swaths was about half that from light swaths. Rain on alfalfa of 40%

moisture or less sometimes caused slightly greater leaching of plant nutrients or 15% greater leaf loss when compared to rain on alfalfa of 65% moisture.

Macerated alfalfa exposed to rain had more than three times the DM loss found in mechanically, or mechanically and chemically conditioned alfalfa. Loss was about 30% CP and 70% other soluble constituents which greatly increased NDF, ADF and ADIP concentrations and reduced CP concentration and IVDMD.

VALUE-ADDED PRODUCTS FROM FORAGE BASED ON SOLID-LIQUID SEPARATION

R.G. KOEGEL, R.J. STRAUB and J.Y. KIM

Introduction

Two processes based on solid-liquid separation have yielded products of economic value: (1) recovery of the fibrous fraction from dairy waste for use as bedding, feed, fuel, or soil amendments, and (2) production of protein concentrates from the juice of freshly cut forage.

Materials and Methods

In past research, presses have been developed which have very successfully carried out both processes. These designs were based on moisture expression trials of the specific material followed by modifications to improve performance. However, when it becomes necessary to design solid-liquid separators for other substances, it would be helpful to have a general mathematical dewatering model based on easily obtained material physical parameters. Progress has been made on such a model during the past year.

Results and Discussion

A number of possibly useful physical parameters have been determined for a variety of manure slurries plus fresh plant material macerated in four different ways. These parameters include "equilibrium moisture" as a function of applied pressure, permeability as a function of dry matter density, and particle size distribution. Several forms of the mathematical model have been proposed and tried to determine their adequacy. While no model, to date, is considered adequate, work will continue until an adequate dewatering model, based on easily-determined physical parameters, is achieved as an aid to solid-liquid separator design.

In addition to the modeling effort, an additional year's experience with a prototype dairy waste separator has been obtained. A second, larger version of this separator has been built and partially evaluated and is scheduled to be used with several other substances such as sewage sludge, cannery waste, and anaerobic digester effluent.

Characteristics and dimensions of three reciprocating separators are shown in Table 1 while performance of the smallest press is shown in Table 2.

Table 1. Dimensions and characteristics of three presses.

	r_i (cm)	r_0 (cm)	r_0/r_i	t (cm)	A (cm^2)	R
Press #1	3.7	7.3	1.97	3.6	124	.68 K'
Press #2	5.7	10.1	1.77	4.4	218	.57 K'
Press #3	8.4	12.7	1.51	4.3	285	.41 K'

Table 2. Performance of press #1.

	INFLUENT			EFFLUENT			FIBER	
Input Kg/min	Solids %	Solids Kg/min	Output Kg/min	Solids %	Solids Kg/min	Output Kg/min	Solids %	Solids Kg/min
210.0	8.83	18.54	193.5	7.06	13.66	16.54	28.56	4.74

MICROBIAL INOCULANT OR PROPIONIC ACID TREATMENT FOR PRESERVATION OF ALFALFA SILAGE FED TO LACTATING DAIRY COWS

G. A. BRODERICK, D. B. RICKER and N. VOLLEBREGT

Introduction

Yield and quality characteristics of alfalfa make it the principal legume forage grown to feed dairy cattle in the U.S. There is a strong trend in dairy production toward increased feeding of alfalfa silage and reduced feeding of alfalfa hay. However, recent work has shown that cows fed all alfalfa silage diets containing 21% CP yielded more milk and milk protein when abomasally infused with casein (Dhiman and Satter, J. Dairy Sci. 72 (suppl. 1): 299, 1989). Feeding cows only wilted alfalfa silage treated with formic acid increased production of milk and milk protein by 3.4 and .11 kg/day compared to feeding cows untreated silage (Nagel and Broderick, Proc. 20th Biennial Conf. on Rumen Function, Vol. 20: No. 36, 1989). Extensive breakdown of alfalfa protein after ensiling at least partly accounts for these protein responses. A lactation trial was conducted to determine if preserving low DM or high DM alfalfa silages with a commercially available microbial inoculant or with propionic acid would reduce protein breakdown in the silo and would improve silage quality and milk production.

Materials and Methods

Third-cutting alfalfa was wilted to 36% DM (LDM) and ensiled with or without a commercial microbial inoculant (MI; Modified Haylage Mate from Cargill Corp., Elk River, MN), or wilted to 61% DM (HDM) and ensiled with or without a commercial preservative applied at 2 kg propionic acid (PA; Storage Mate I from Cargill Corp., Elk River, MN)/ton (wet basis). Twenty-four cows averaging 28 days in milk were fed four diets based on these silages in a 4X4 Latin square. Each diet contained (DM basis) 80.0% alfalfa silage, 18.8% high moisture corn, plus vitamins and minerals, and averaged 18.7% CP, 25% ADF and 1.48 Mcal NE_l/kg. Each cow was fed its respective diet with the appropriate alfalfa silage for 3-week periods before switching to the next silage (total 12 weeks). Milk production data from week 1 were discarded, and the data analyzed were from the last 2 weeks of each period. Milk production was measured at each milking; milk samples were analyzed for fat, protein, lactose, SNF and urea. Cows were weighed on three consecutive days at the start of the trial and at the end of each period. Four hours after feeding on day 20 of each period, blood samples were taken from the tail vein of each cow and blood plasma was analyzed for glucose and urea. Also on day 20, rumen samples were taken at 0, 1, 2, 3, 4, and 6 hours after feeding from four cannulated cows fed the same diets and analyzed for pH, ammonia and total amino acids (TAA).

Results and Discussion

A summary of silage temperatures, DM recoveries and analyses is in Table 1. Lower pH, ADIN, ADF, NDF, ash ($P<.001$), and silo temperatures ($P<.06$) were found in LDM vs. HDM silages; CP content and DM recovery were higher ($P<.001$) in LDM than HDM silage. Treatment of LDM alfalfa silage with MI resulted in 2.8 percentage units less NPN and a mean 6°C reduction ($P<.01$) in silo temperature. There was reduced protein breakdown in the silo with the HDM silages, and HDM silage treated with PA contained 2.7 percentage units less NPN.

Intake of DM, weight change, production data, blood glucose and milk and blood urea are in Table 2. Feeding of diets with LDM alfalfa silage resulted in DM intakes which were .9 kg/day greater (P<.001) than that with HDM silage; neither MI or PA treatment altered DM intake. Body weight was not affected by silage source. Production of milk, fat, protein, lactose and SNF on LDM silages was increased by 12, 9, 14, 12 and 13%, respectively, over that on HDM silages. Feeding LDM treated with MI increased (P<.05) protein secretion by 30 g/day and resulted in trends for increased production of milk (P<.10), lactose (P<.10) and SNF (P<.07).

Overall rumen pH and acetate-propionate ratio were lower on LDM than HDM alfalfa silage (P<.001), suggesting higher rumen fermentability of the LDM silage. Lower rumen concentrations of branched VFA overall and ammonia at 2 hours after feeding implied lower rumen degradation of protein in HDM silages. Treatment of LDM with MI silage also reduced rumen ammonia (P<.08) and TAA (P<.03) at 2 hours after feeding and may have been due to reduced protein degradation.

Summary and Conclusion

Feeding third-cutting alfalfa silage harvested at about 36% DM (the LDM silages) reduced nutrient losses and increased yields of milk and milk components by 9-14%, when compared to silage harvested at about 61% DM (the HDM silages). Treatment of the LDM alfalfa silage with a commercial microbial inoculant reduced silage NPN, may have reduced rumen protein degradation, and increased production of milk, lactose, SNF, and especially protein. LDM silage had somewhat greater protein degradation than HDM silage; treatment of HDM silage with a propionic acid-containing preservative did not improve its utilization.

Table 1. Mean silage analyses.

Item	Silo A1	A2	A3	A4	SEM	STATS Prob. Tr effect	Contrasts A1 vs.A2	A3 vs. A4	LDM vs. HDM
DM, %	37.3	35.0	57.9	60.4	.3	<.001	<.001	<.001	<.001
Temp, C	38.4	32.5	39.1	40.4	1.4	.010	.006	.490	.061
pH	4.60	4.72	5.80	5.73	.09	<.001	.337	.598	<.001
TKN, % DM	2.94	2.99	2.86	2.94	.02	<.001	.094	.004	.001
ADIN, % TKN	8.00	7.94	12.17	13.51	.60	<.001	.945	.130	<.001
NPN, % TKN	56.0	53.2	49.6	47.0	.9	<.001	.038	.050	<.001
NH3-N, % TKN	10.5	12.0	12.7	12.5	.3	<.001	.001	.609	<.001
TAA-N, % TKN	39.2	35.4	19.2	16.7	.9	<.001	.009	.071	<.001
Unidentified-NPN, % TKN	6.3	5.8	17.8	17.7	.7	<.001	.584	.950	<.001
ADF, % TKN	37.1	37.6	43.7	43.0	.66	<.001	.636	.446	<.001
NDF, % TKN	43.6	44.3	52.0	51.7	.80	<.001	.562	.734	<.001
Ash, % TKN	10.31	10.20	10.84	10.96	.07	<.001	.290	.249	<.001
DM Recovery, %	92.7	93.7	90.2	89.2	.6	<.001	.260	.267	<.001

Silo A1 = LDM Control; A2 = LDM treated with microbial inoculant; A3 = HDM Control; A4 = HDM treated with propion acid; TKN = total Kjeldahl N.

Table 2. Dry matter intake, BW change, production of milk and milk components, blood glucose, and milk and blood urea.

Item	Silo A1	A2	A3	A4	SEM	STATS Prob. Tr effect	Contrasts A1 vs.A2	A3 vs. A4	LDM vs. HDM
DM intake, kg/day	19.60	19.42	18.60	18.63	.24	.003	.580	.910	<.001
BW change, kg/day	-0.11	-0.03	-0.32	-0.08	.11	.289	.618	.137	.255
Milk, kg/day	27.78	28.70	25.42	25.12	.40	<.001	.093	.574	<.001
Fat, %	3.30	3.25	3.35	3.31	.04	.402	.423	.467	.186
Fat, kg/day	.912	.931	.852	.836	.018	.001	.459	.540	<.001
Protein, %	2.81	2.83	2.78	2.76	.01	<.001	.259	.259	<.001
Protein, kg/day	.776	.806	.703	.689	.011	<.001	.043	.352	<.001
Lactose, %	4.81	4.82	4.80	4.78	.01	.152	.879	.252	.047
Lactose, Kg/day	1.334	1.379	1.219	1.197	.020	<.001	.099	.409	<.001
SNF, %	8.25	8.28	8.19	8.15	.02	<.001	.152	.053	<.001
SNF, kg/day	2.282	2.363	2.078	2.040	.032	<.001	.067	.397	<.001
Milk/DM intake	1.43	1.49	1.37	1.35	.03	.418	.369	.159	.909
Plasma glucose, mg/dl	68.1	66.6	68.1	69.3	.7	.123	.185	.257	.093
Milk urea, mM	5.25	5.55	4.64	4.85	.15	<.001	.139	.294	<.001
Plasma urea, mM	7.87	7.97	7.56	7.56	.10	.005	.464	.985	<.001

Silo A1 = LDM Control; A2 = LDM treated with microbial inoculant; A3 = HDM Control; A4 = HDM treated with propionic acid; TKN = total Kjeldahl N.

INOCULATION OF ALFALFA SILAGE WITH LACTOBACILLUS PLANTARUM

T.R. DHIMAN, C.M. WACEK and L.D. SATTER

Introduction

This study was part of a series of lactation studies at the U.S. Dairy Forage Research Center designed to measure the milk production response to feeding of alfalfa silage that had been inoculated with lactic acid bacteria (LAB). It appears that the potential response in milk production is small, often smaller than a statistically significant response would be, given the number of lactating cows that are typically available for experimentation. However, the slight increase in milk production which is often seen may be a profitable increase, even though a claim cannot be made that the response is statistically significant. Therefore, a series of repeating studies was undertaken to obtain a realistic estimate of the potential impact of silage inoculation on milk production.

Materials and Methods

Second crop alfalfa cut at mid bud was field wilted to 44-49% DM and ensiled as control or inoculated silage. The inoculant (Ecosyl) was supplied by Imperial Chemical Industries (ICI) and contained Lactobacillus plantarum. The inoculant was applied at the rate of 10^5 CFU per gram of forage. Thirty-two mid lactation Holstein cows were randomly assigned to two groups (Control and Treatment) of sixteen each according to their milk yield. The experimental design was a simple switch back with two periods of six weeks each. The diets contained (DM basis); 65% alfalfa silage, 23.2% high moisture ear corn, 10% roasted soybeans, 1.1% dicalcium phosphate, .7% trace mineral salt and a trace amount of vitamin ADE supplement. Chemical composition of the silage is shown in Table 1. Daily feed offered as a total mixed ration and feed refusals were recorded. Daily milk yield was measured. Milk samples were collected on two consecutive milkings and analyzed for fat, protein, lactose and somatic cells. Body weights were measured two days in a row at the start and end of each period and once weekly during the experimental period. Feed dry matter determination was done on weekly com-

posite samples of the total mixed ration, feed refusals and silage. Dry matter content of other feed ingredients was measured on samples taken once weekly. Silage analysis was performed on samples taken from silos once weekly during the lactation experiment.

Results and Discussion

Use of the silage inoculant reduced pH, ammonia, succinate, and acetate contents of treated silage compared to control silage (Table 1), indicating more rapid fermentation and decreased protein breakdown in inoculated silage. The lactation results are shown in Table 2. Feeding inoculated silage did not statistically affect milk yield, fat, protein and lactose percentage in milk although there was a small but insignificant increase in milk production. Dry matter intakes were similar for the control and treatment groups; however, there was a slight increase in body weight gain with cows fed inoculated silage.

Conclusion

Based on the results of the present experiment, inoculation of alfalfa silage with Lactobacillus plantarum (Ecosyl) did improve the silage fermentation, but the small positive effect on milk production was not statistically significant.

Table 1. Chemical composition of control and treated silages (% DM basis).

Item	Silage	
	Control	Treated
Dry matter	42.6	44.3
Crude protein	18.0	18.5
NDF	41.1	41.2
ADF	33.4	33.4
pH	4.54[a]	4.41[b]
Ammonia N, % of total-N	6.7[a]	4.6[b]
Free amino acid N, % of Total-N	24.3	21.5
Succinate, mg/gDM	4.03[a]	2.60[b]
Lactate, mg/gDM	49.8	52.3
Acetate, mg/gDM	24.1[a]	15.2[b]

[ab] Means in the same row with different superscripts differ (P<.05).

Table 2. Effect of silage inoculation on lactation performance.

Item	Control	Treatment	SE
Milk yield, kg/d	28.9	29.4	.8
3.5% FCM, kg/d	28.7	28.9	.8
Fat, %	3.47	3.42	.07
Protein, %	2.98	2.98	.03
Lactose, %	4.76	4.77	.04
Fat yield, kg/d	1.0	1.0	.03
Protein yield, kg/d	.86	.88	.02
Somatic cell count	235	181	96
DM intake, kg/d	23.6	23.9	.5
Body wt change during experimental period, kg	17.2	25.4	3.2

No significant difference for any parameter (P<.05).

ADDITIVE EFFECTS ON IN VITRO DIGESTION KINETICS OF GRASS SILAGE

E.M.G. THORSTENSSON, D.R. BUXTON, P. LINGVALL, A.K. GUSTAFSSON and C. RAMMER

Introduction

Cell walls (CW) of forages differ in digestibility depending on their structure and composition. Neutral detergent fiber (NDF), a good estimate of CW concentration, can be divided into potentially digestible NDF (PDNDF) and indigestible residue (IR). Forage quality could be improved by increasing concentration and digestion rate of PDNDF. Proper ensiling, when harvesting forage at low dry matter (DM) concentrations (<30% DM), often requires addition of silage additives to achieve desired fermentation and forage digestibility. The objectives of this study were to evaluate the effects of formic acid, inoculants, and CW degrading enzymes on CW concentration and composition and on in vitro digestion kinetics of CW of timothy (*Phleum pratense* L.)-orchardgrass (*Dactylis glomerata* L.) silages.

Materials and Methods

Orchardgrass and timothy were grown in a mixture at Kungsangen's Research station at the Swedish University of Agricultural Sciences. This grass material was harvested at 25% DM to be used in two experiments. In *Exp. 1,* herbage was ensiled in 2-kg silos for 100 days and treated with the following additives: Formic acid (4 liters T^{-1} fresh herbage; Perstorp AB, Sweden), Siloferm (inoculant, consisting of 70% *Pediococcus acidilactici* and 30% *Lactobacillus plantarum,* 10^6 LAB g^{-1} fresh herbage; Arla, Sweden) *Pediococcus* bacteria (10^6 LAB g^{-1} fresh herbage; Arla, Sweden), Econase (2 endo-1,4-b-glucanase units (ECU); Alko, Finnland), and the combination Siloferm+Econase (2 and 4 ECU). One ECU produces one nmole of reducing sugars, as glucose, in one second. One thousand colonial forming units (CFU) of *Clostridium tyrobutyricum* g^{-1} fresh herbage were added to all treatments including the control. In *Exp. 2,* herbage was ensiled in 10 kg silos for 100 days. Formic acid (4 liters T^{-1} fresh herbage) and Siloferm (10^6 LAB g^{-1} fresh herbage) were added to the herbage before ensiling.

Silage samples from both experiments were incubated in rumen fluid and NDF concentration was determined at appropriate fermentation times. Data were fitted with a first-order, nonlinear digestion model to estimate concentrations of PDNDF and IR, digestion rate of PDNDF, and lag time before digestion. At zero time, NDF, ADF, and ADL concentrations were sequentially analyzed on undigested samples. Hemicellulose and cellulose concentrations were calculated as weight losses between these concentrations.

Results and Discussion

Exp. 1. Silage treated with formic acid had lower NDF, hemicellulose, cellulose, PDNDF, and IR concentrations and a faster digestion rate than the control (Table 1). Addition of Econase resulted in significantly lower initial NDF, cellulose, and PDNDF concentrations than in silage treated with Siloferm alone, Pediococcus, or the control. Siloferm and Pediococcus, which had similar effects, had smaller effects on CW than formic acid or Econase. These effects may result from LAB fermenting sugar to lactic acid and leaving fibers undegraded. These inoculants generally resulted in greater NDF, hemicellulose, cellulose, and PDNDF concentrations than when formic acid or Econase was used. Silage treated with Pediococcus had a faster digestion rate than the control. Siloferm in combination with Econase (2 ECU) increased PDNDF concentration compared with Econase alone. Econase in combination with Siloferm reduced NDF and cellulose

concentrations compared with Siloferm alone. When the concentration of Econase was doubled in combination with Siloferm, NDF, cellulose, and PDNDF concentrations were significantly lowered. The main reason for these changes in Econase treated silage is that the enzymes in the additive degraded the digestive portion of the CW during the ensiling process.

Exp. 2. Formic acid treated herbage had lower NDF, cellulose, and PDNDF concentrations than control and Siloferm treated herbage, which behaved similarly regarding CW concentration and digestion (data not shown). This result is consistent with data from Exp. 1. The reason for lower CW concentration of formic acid treated silage may be that formic acid hydrolyzes CW components.

In summary, both formic acid and Econase reduced NDF concentration and improved silage digestibility.

Table 1. Neutral detergent fiber (NDF), acid detergent lignin (ADL), hemicellulose, cellulose, potentially digestible NDF (PDNDF), and indigestible residue (IR) concentrations; digestion rate; and digestion lag of orchardgrass-timothy silage. Data are average of two replicates from *Exp. 1.*

Treatments[1]	NDF	ADL	Hemicellulose	Cellulose	PDNDF	IR	Digestion rate	Digestion lag
	g kg^{-1} dry matter						h^{-1}	h
Control	518^{a}	28^{a}	197^{a}	289^{c}	369^{a}	149^{a}	.096^{a}	4.2^{a,b}
Formic acid, 4 L T^{-1}	491^{b}	25^{a,b}	185^{b,c}	270^{a}	357^{b,c}	134^{b}	.126^{b}	4.0^{a,b}
Siloferm, 10^{6} LAB[2] g^{-1}	510^{a}	26^{a,b}	196^{a,c}	282^{b}	371^{a}	140^{b}	.110^{a,b}	4.2^{a,b}
Pediococcus, 10^{6} LAB g^{-1}	510^{a}	23^{b,c}	202^{a}	280^{b}	373^{a}	136^{b}	.124^{b}	4.7^{a}
Econase, 2 ECU[3] g^{-1}	489^{b}	28^{a}	188^{a,b}	268^{a}	348^{b}	142^{a,b}	.096^{a}	4.3^{a,b}
Siloferm+Econase, 2 ECU g^{-1}	496^{b}	25^{a,c}	192^{a,b}	270^{a}	361^{a,c}	135^{b}	.106^{a,b}	3.4^{b}
Siloferm+Econase, 4 ECU g^{-1}	473^{c}	26^{a,c}	182^{b}	257^{d}	333^{d}	140^{b}	.119^{b}	4.2^{a,b}

[1]10^{3} colonial forming units of *Clostridium tyrobutyricum* were added to all treatments.
[2]LAB: lactic acid bacteria.
[3]ECU: endo-1,4-b-glucanase unit.
$^{a-d}$Treatment means with different superscripts differ significantly at 10% probability.

EFFICACY OF BACTERIAL INOCULANTS FOR ALFALFA SILAGE

L.D. SATTER, R.E. MUCK, B.A. JONES, T.R. DHIMAN, J.A. WOODFORD and C.M. WACEK

Introduction

Silage additives are marketed with claims that they improve silage quality and animal performance. Bacterial inoculants are the most common additives in North America and are considered by some in Northern Europe as a replacement for direct acidification of silage. This report summarizes research at the U.S. Dairy Forage Research Center utilizing bacterial inoculants as an ensiling aid for alfalfa.

Results and Discussion

The first objective was to determine the influence of bacterial inoculants and substrate addition to alfalfa ensiled at different dry matter contents. Field wilted alfalfa was chopped with a forage harvester at 33, 43 and 54% dry matter, treated and ensiled in laboratory silos during four cuttings in each of two years. Treatments were control (C); sugar addition at 2% of fresh weight (S); inoculum applied at $3x10^5$ bacteria/g herbage (I); and sugar plus inoculum (IS). Duplicate silos were opened and analyzed after 1, 2, 3, (4 or 5), 7, 14 and 60 days of fermentation. The initial rate of proteolysis of alfalfa protein decreased with increasing DM content of the alfalfa, and was not influenced by the year, cutting or silage treatment. Inoculation increased ($P<0.05$) the rate of pH decline for all silage dry matters and shortened the lag time prior to pH decline with 33 and 43% dry matter silages. Sugar addition had no effect on rate of pH decline or lag time. Inoculation and sugar addition both lowered final pH, acetic acid, ammonia (NH_3), free amino acids (FAA) and soluble non-protein N (NPN) in silages ($P<0.01$) and increased lactic acid content with 33 and 43% dry matter silages. Only inoculation was beneficial at 54% ($P<0.01$) with no difference between I and IS. Sugar addition increased peptide N at 33% DM, but inoculation consistently increased ($P<0.01$) peptide N with all DM treatments. With low dry matter (30-37% DM) silage, effectiveness of both sugar and inoculant additions for lowering pH and increasing the lactate:acetate ratio was related to buffer capacity and the ratio of sugar:buffer capacity. Treatment effectiveness with high dry matter silage was influenced by the number of epiphytic lactic acid bacteria (LAB) and not substrate availability. Reduction of NPN, NH_3 and FAA in the silage by the treatments was not correlated to any of the forage characteristics and was influenced only by moisture content of the silage.

The second objective was to measure the benefit, if any, to animals consuming silage that was treated with a bacterial inoculant. A total of 17 comparisons between control and treated silages was made in a series of 11 experiments with lactating cows. Silage additives rather consistently improved silage fermentation, but this did not always result in improvements in dry matter intake or milk production. In order to get a response in animal performance, the inoculation had to increase the number of LAB on the crop at ensiling by a factor of 10 or more. If the animal response is averaged for just those studies where the number of lactic acid bacteria were successfully increased ten fold or more by the inoculant, then a 2.5% increase in milk production was measured. This represents a return of $2.50 for every $1.00 invested in an inoculant. If inoculants are used indiscriminately, then the average milk production response drops to 1.2%, or a return of about $1.20 for every $1.00 invested. Lactating cows fed treated silage tended to gain slightly more weight than control cows in 11 of the 13 comparisons made.

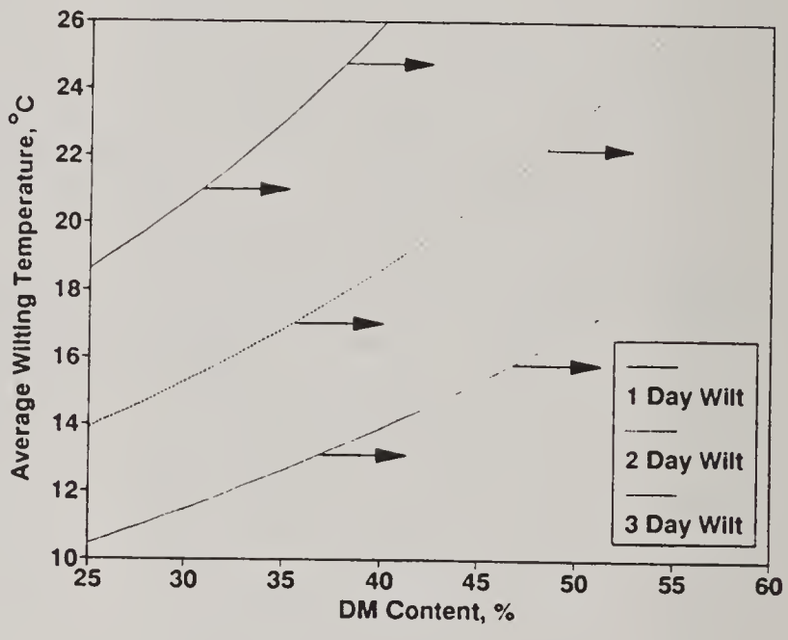
The third objective was to determine what factors influenced the number of epiphytic LAB present at the time of ensiling. Numbers of LAB were measured through-

out the growing season over a 5 year period. Moisture content, machinery records, weather data and crop records such as dry matter yield and swath dimensions were kept for correlation with LAB numbers. Periodically, samples of alfalfa before and after mowing and immediately prior to chopping were analyzed for LAB.

Numbers of LAB in the standing crop were always below detectable levels (<10 CFU/g). Immediately after mowing, low levels of LAB, averaging 51 CFU/g, were found on over half the samples. As a result, the LAB population on chopped lucerne was found to be related to growth of LAB in the swath during wilting or to "apparent" chopper inoculation. In both cases, LAB numbers were correlated to wilting time and average wilting temperature. Growth in the swath was also positively correlated with moisture content of the lucerne at harvest whereas "apparent" chopper inoculation was increased by rainfall during wilting.

Regression equations developed from the first two years of the study successfully predicted LAB numbers in the third year at our research farm. The equations were further tested at five additional farms around Wisconsin in the fourth and fifth years with nearly equal success. Only the first field harvested in the spring and alfalfa harvested above 60% DM after 1 day of wilting were consistently over-predicted.

Using the results generated from the latter two objectives, a graph of conditions producing economic benefits from inoculant usage can be developed. Assuming a $2.50 return for every $1.00 invested in inoculant when the epiphytic population is 10% or less of the inoculant LAB, then an inoculant supplying 10^5 CFU/g should be used when the probability of the epiphytic population being 10^4 CFU/g or less is 40% (i.e., 1/2.5) or greater. A potential for profitable utilization of a silage inoculant exists when conditions fall to the right of the appropriate line in the figure below.



Conclusions

Benefit from silage inoculants seems to occur when the inoculant can increase the number of lactic acid bacteria in the silage by a factor of 10 or more. This is likely to occur when the average wilting temperature is below 14-18°C and the wilting period is relatively short.

EFFECTS OF GRASS COMPOSITION ON SILAGE FERMENTATION AND AEROBIC STABILITY

R.E. MUCK, S.F. SPOELSTRA and P.G. VAN WIKSELAAR

Introduction

Particularly in horizontal silos, gas composition changes with storage time. During active fermentation, the gas atmosphere is often 90% or more CO_2. After active fermentation, CO_2 will drain from a silo and be replaced by air. Because the oxygen will be respired, the gas composition gradually becomes 80% N_2-20% CO_2. High levels of CO_2 have been reported to inhibit many fungal strains which suggests that high CO_2 levels during fermentation and storage may lower yeast and mold numbers and make the silage less susceptible to heating and aerobic losses. The objective of this study was to ensile whole plant corn under two different gas atmospheres (100% CO_2 and 80% N_2:20% CO_2) and determine effects on various microbial groups and on subsequent aerobic stability.

Methods

Three different experiments were performed: one with fresh or fresh-frozen chopped, whole plant corn; the other two with re-ensiled corn silage.

Experiment 1. Four similar trials were performed. Two 10 l laboratory silos with sampling ports were each filled with 2.5 kg of fresh-cut or thawed, fresh-frozen corn. One silo was flushed with 100% CO_2 (CO_2 treatment), the other with a gas mix of 80% N_2 and 20% CO_2 (80/20 treatment). Samples from each silo were taken on days 0, 1, 2, 3, 4, 7 and 11 and once weekly thereafter and analyzed for pH, chemical constituents and microbial groups (lactic acid bacteria, enterobacteria, bacilli, acetic acid bacteria, yeasts, molds). The total incubation period was eight weeks. During sampling the gas composition in the silos was measured. When gas composition deviated substantially from the desired levels, the silos were flushed with the appropriate gas mixture. After eight weeks of anaerobic incubation, the silos were opened, completely aerated and then closed again. Aerobic stability was measured by thermocouple and periodic measurement of the oxygen content. Daily samples for chemical and microbial analysis were taken until heating occurred.

Experiment 2. Four 700 l silos were filled with 4-month old corn silage. Two silos had 2.5 kg dry ice placed on the bottom before filling (CO_2 treatment). The other two silos were just filled. The silos were sealed and had a water lock on top to allow gas to escape. After 0, 1, 2 and 3 months of incubation, samples of approximately 10 kg per silo were taken. Approximately 5 kg of corn silage were put in an insulated, 5-cm thick wall, polystyrene foam box (28x22x26 cm). Temperature inside the box was measured with a thermocouple to determine the initiation significant of aerobic deterioration (1°C rise above ambient). Samples at each opening were taken for chemical and microbial analysis as in Experiment 1.

Experiment 3. Five-month old corn silage was re-ensiled in two 10 l laboratory silos. One silo was flushed and maintained with a 100% CO_2 atmosphere; the other was flushed and kept at 80% N_2:20% CO_2. These silos were sampled weekly. After 6 weeks incubation, silages were aerated and aerobic stability measured as in Experiment 1. Samples from the anaerobic and aerobic phases were analyzed for the same chemical and microbial constituents as in Experiment 1.

Results

Experiment 1. Silage fermentation was not significantly affected by gas composition in any of the four trials. Numbers of lactic acid bacteria and enterobacteria

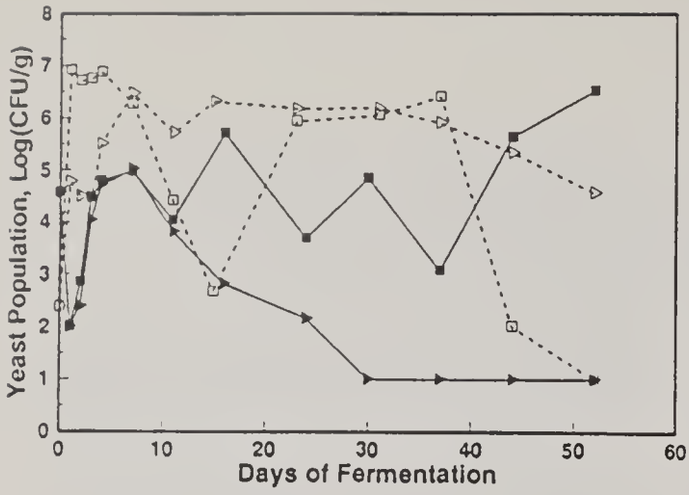


Figure 1. Yeast population in whole plant corn silage during fermentation as affected by gas composition.

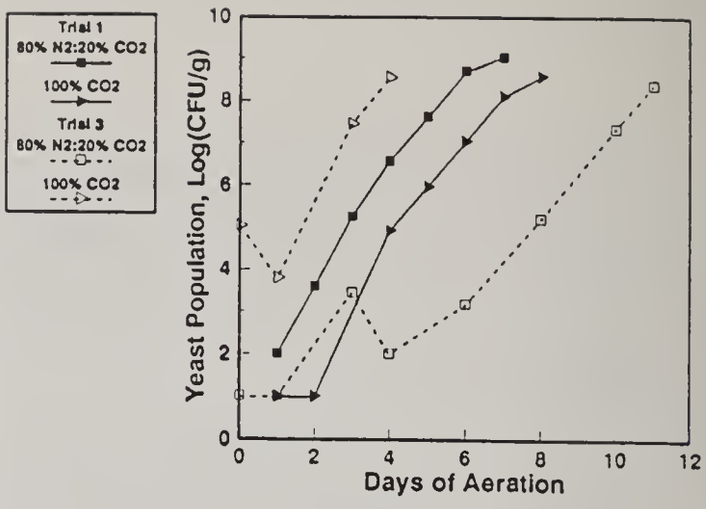


Figure 2. Yeast population in whole plant corn silage after exposure to air as affected by gas composition during fermentation.

were similar between both treatments. Bacilli tended to be higher in the 100% CO_2 treatment, but the observed differences had no apparent affect on overall fermentation. Silage chemical composition (pH, lactic and acetic acids, ethanol) was not significantly different between treatments at any point during fermentation in any of the four trials.

Yeast counts were different between the two treatments, but not consistently so (Figure 1) and resulted in different aerobic stabilities (Figure 2). In two trials, the 100% CO_2 treatment had the lower yeast counts and therefore the greater aerobic stability. The reverse was true in the other two trials.

Experiment 2. One month after re-ensiling, the dry ice treated silages had lower yeast counts and were more stable than the silage at re-ensiling. In the succeeding two months, yeasts counts gradually increased so that after 3 months re-ensiling the treated silage tended to be (although not significantly) less stable than the material entering the 700 l silos. In the untreated silages, yeast counts rose in the first month most likely due to air trapped in the silage at re-ensiling. This reduced stability compared with the original silage. Yeast counts and aerobic stability were not further affected in later samplings.

Experiment 3. No effect of treatment was found on lactic acid bacteria and bacilli counts, pH and aerobic stability. Yeast counts were higher in the 80% N_2:20% CO_2 atmosphere, but the dominant strain(s) at opening was not ultimately responsible for significant heating.

Conclusions

The level of CO_2 in the silo atmosphere has little effect on silage fermentation. It had been anticipated that high levels of CO_2 would reduce yeast counts during silo storage and thus improve aerobic stability. This was observed in several trials; however, in other trials yeast counts were higher in the 100% CO_2 treatment leading to reduced stability. Apparently there are strains of yeast that thrive under high CO_2 conditions and can predispose a silage to heating. Therefore maintenance of high CO_2 levels in the silo does not appear to be of any consistent benefit for maintaining silage quality and reducing aerobic losses.

MODELING OF FUNGAL GROWTH IN AERATED SILAGES

R.E. PITT and R.E. MUCK

Introduction

The largest losses of dry matter and energy from crops stored in silos are usually due to microbial respiration at feed out or from the slow infiltration of oxygen into the silo during storage. Most microbial respiration in silages has been linked to fungi (both yeasts and molds), but little is known regarding the principal factors that affect their development in silage. Furthermore, many silage additives make claims of improved aerobic stability, but research trials have often found inconsistent responses that are difficult to interpret. The complexity of the problem suggested that a computer model of fungal growth in silage might help determine those factors or management strategies of greatest importance in reducing fungal growth and respiration.

Methods

A simulation model of mesophilic and thermophilic yeasts and molds was developed in PASCAL for use on a microcomputer. The growth of the four groups of fungi were assumed to be affected by temperature, pH, water activity, lactic, acetic and propionic acids. Empirical and theoretical relationships describing the effects of these factors on growth and maintenance requirements were developed from literature data. Substrates used by the fungi, in order of preference, were water soluble carbohydrates, ethanol, lactic acid and acetic acid. Gas movement and heat transfer were not considered in the model. The silage was assumed to be well aerated so that oxygen and carbon dioxide levels were the same as atmospheric conditions. The energy to raise silage temperature was set at a constant percentage of that released by fungal respiration.

The model was validated using published data from experiments with corn, grass and legume silages. A sensitivity analysis was performed to investigate the effects on aerobic stability of fungal levels, initial temperature, pH, moisture content, substrate concentrations and pre-ensiling forage composition and moisture content. Finally, the model was used to investigate the effects of silage additives (bacterial inoculants, sugars, amylases, formic acid and propionic acid) on aerobic stability.

Results

The fungal model explained 82% of the variation in aerobic stability data for highly aerated grass and corn silages where all major inputs were known. The model did not perform as well in some alfalfa silages where the presence of a fungal inhibitor other than fermentation acids was apparently present.

According to the model, silages begin heating or become unstable when fungal concentrations reached 0.001 g/g silage. The relative importance of yeasts or molds depended primarily on their initial concentrations in the silage upon exposure to air.

The length of aerobic stability was found to be most affected by temperature, water soluble carbohydrate concentration, fungal populations and acid concentrations in interaction with pH. Moisture content, pH and heat loss percentage were of lesser importance.

Porosity and its effect on oxygen movement were not considered in this model and are often thought to be the principal factors predisposing drier silages to aerobic deterioration. However, the model indicates that when silages are well aerated, low moisture silages will be less stable than wetter silages primarily because of low concentrations of fermentation acids along with high pH. Thus, there are several reasons for greater instability in drier silages.

Increasing water soluble carbohydrate content at ensiling promotes aerobic stability as long as there are no residual sugars because the additional fermentation acids produced inhibited fungal growth. However, excess sugar may reduce stability because the fungi grow approximately twice as fast on sugar as on ethanol, lactic acid or acetic acid. As a result, sugar or enzyme additives may improve or reduce aerobic stability based on the changes in composition of the silage after fermentation.

Bacterial inoculants also may improve or reduce aerobic stability (Figure 1). Such variation is due to subtle shifts in fermentation products and pH. At a given pH, lactic acid is less inhibitory than acetic acid. Inoculants generally shift fermentation from acetic toward lactic acid. This alone would reduce stability; however, pH is usually lowered, increasing the inhibitory activity of both acids. Therefore, effects in either direction are possible depending on circumstances.

Propionic acid was the only silage additive that consistently improved stability (Figure 2). Propionic acid is inhibitory to yeasts and molds so that increasing concentrations directly reduce fungal growth rate.

Overall, the model appears to be reasonably accurate and provides insights into the efficacy of various practices and silage additives. Further modeling work is needed to link the fungal growth model to models of heat and gas transfer in the silo. This will permit modeling of aerobic losses and heating within the silo.

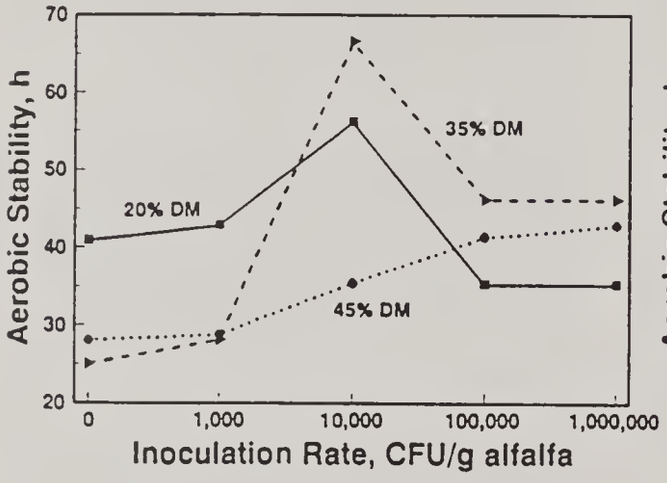


Figure 1. Predicted effect of inoculation on the aerobic stability of alfalfa silage with an initial fungal concentration of 10^{-8}g/g DM.

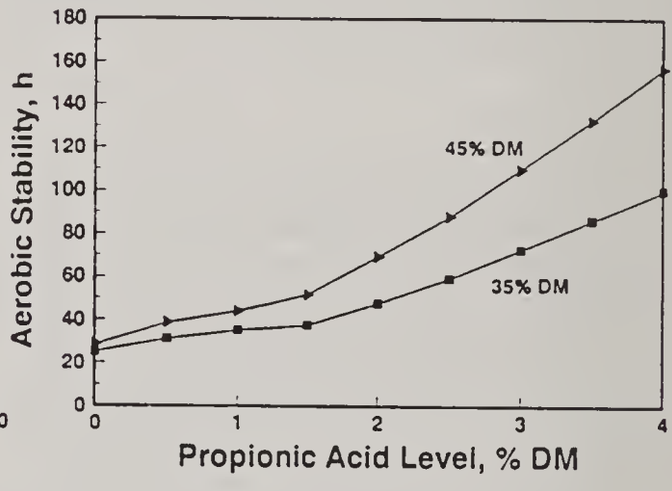


Figure 2 Predicted effect of propionic acid addition on the aerobic stability of alfalfa silage with an initial fungal concentration of 10^{-8}g/gDM.

CONTINUOUS CULTURE OF THE RUMINAL BACTERIUM *Ruminococcus flavefaciens* ON CELLULOSE

P.J. WEIMER, Y. SHI and C.L. ODT

Because cellulose is the major dietary component of forage-fed ruminants, the activities of the cellulolytic microbial population of the rumen are of central importance to the nutrition and productivity of the whole animal. Much of our information on the physiology and biochemistry of ruminal cellulolytic bacteria has come from batch culture studies or from continuous culture studies employing soluble carbohydrate substrates. Continuous culture studies with insoluble substrates (viz. cellulose) have been limited by the inherent difficulty of continuously and accurately delivering these substrates to culture vessels. The objective of this project was to develop a practical device for continuous culture of anaerobic cellulolytic microorganisms on cellulose-containing media, and to use the device for quantitative studies of cellulose fermentation by ruminal cellulolytic bacteria.

Materials and Methods

A continuous culture system capable of delivering insoluble substrates was designed and constructed. The system consisted of three main components: i) a sterile 8 l glass microcarrier reservoir in which the medium containing microcrystalline cellulose particles of relatively small size (< 45 um) was well-mixed through the combined use of overhead-drive mechanical stirring and a rapid sparge of CO_2 through a ceramic gas diffusion rod; ii) a peristaltic delivery system in which the medium was delivered through narrow-bore polyvinylchloride tubing as small (~20 ul) discrete liquid segments separated by intervening bubbles of CO_2; and iii) a stirred, temperature-controlled two-liter fermentor with a working volume of 940 ml. During operation of the delivery system, the high surface tension of the aqueous medium permitted retention of the cellulose in the liquid segments without the settling or plugging problems characteristic of other delivery systems. Using this system, we can easily obtain dilution rates in the range of 0.01-0.25 h^{-1} (corresponding to retention times of 4-100 h) a range somewhat broader than the range of growth rates of ruminal cellulolytic bacteria in vivo.

Ruminococcus flavefaciens FD-1 was grown in this system at 39°C and pH 6.6, at five different dilution rates and a feed concentration of 4.8 g cellulose/l. Culture samples were withdrawn by syringe 1-3 times daily for analysis of fermentation parameters. Residual cellulose was determined gravimetrically following boiling in neutral detergent. Soluble fermentation products were determined by HPLC. Cell mass was estimated from nitrogen content (determined by Dumas combustion and gas chromatography) of cell pellets washed with 0.9 % NaCl; for these calculations, cells were assumed to be 90% organic material by weight, and the organic fraction assumed to have a molecular composition of $C_5H_7O_2N$. Reducing sugars in culture supernatants were determined colorimetrically using the anthrone procedure.

Results

The continuous culture device delivered volumes of medium and masses of cellulose which were constant for each dilution rate and independent reservoir volume. Microscopic examination revealed that, at all dilution rates tested, the cellulose particles were essentially completely colonized by the bacteria (i.e., the culture was cellulose-limited). Passage of five to

seven culture volumes through the fermentor was required to achieve steady state conditions following each dilution rate change.

Fermentation parameters for *R. flavefaciens* FD-1 are shown in Table 1. Increasing dilution rate was accompanied by decreased cellulose conversion, increased cell mass, and a shift in fermentation products toward more acetate and formate, and less succinate. Free reducing sugars remained low ($\leq$ 0.1 mg / L as glucose) at all dilution rates, indicating that cellulose hydrolysis was the rate-limiting step in the cellulose fermentation. The following fermentation parameters were derived from the chemostat data: first-order rate constant for cellulose degradation = 0.11 / h; cell maintenance coefficient = 0.067 g cellulose / g cells / h; maximum growth yield (corrected for maintenance) = 0.24 g cells / g cellulose consumed. Comparison of these values to literature values for another ruminal cellulolytic bacterium, *R. albus*, indicates that *R. flavefaciens* degrades cellulose more rapidly and with a higher cell yield and lower maintenance coefficient. These data are consistent with the purported role of *R. flavefaciens as* one of the major cellulolytic bacteria in the rumen. Comparison of the cell yield values to those reported for cellobiose-limited cultures by other workers suggests that growth on cellulose is particularly efficient (i.e., that the additional energetic costs of a cellulolytic lifestyle are minimal).

Table 1. Fermentation parameters for *Ruminococcus flavefaciens* FD-1 grown in steady-state, cellulose-limited continuous cultures at pH 6.6.

D	Cellulose consumed	Cell yield	mol products/ mol anhydroglucose consumed		
(h^{-1})	(% of amount fed)	(g cells / g cellulose used)	Acetate	Succinate	Formate
0.017	87.4	0.126	88.2	68.8	26.4
0.044	76.2	0.156	82.1	57.6	33.6
0.059	70.6	0.205	88.4	62.7	48.2
0.076	69.7	0.198	87.6	50.4	45.0
0. 101	51.7	0.218	78.9	45.1	51.5

DIFFERENTIAL FERMENTATION OF CELLULOSE ALLOMORPHS BY RUMINAL CELLULOLYTIC BACTERIA

P.J. WEIMER, A.D. FRENCH and T.A. CALAMARI, JR.

In addition to its usual native crystalline form (cellulose I, hereafter designated I), cellulose can exist in a variety of alternative crystalline forms (allomorphs) which differ in their unit cell dimensions, hydrogen bonding relationships, and chain packing schemes. These substrates may be regarded as substrate analogs which permit us to examine the effects of structure on biodegradation at the molecular level. In addition, these allomorphs may also be encountered by microorganisms exposed to pretreated cellulosic substrates since such delignifying agents as sodium hydroxide or ammonia are known to effect these allomorphic conversions. The purpose of this study was to prepare and characterize the different cellulose allomorphs, examine their digestion kinetics by rumen microflora, and to relate the

degradation patterns observed to well-characterized structural models in order to identify structural determinants which have strong effects on cellulose biodegradability.

Materials and Methods

The allomorphs were prepared by modifications of various published chemical treatment procedures: II was prepared from I by alkaline mercerization; III_I was prepared by treatment of I with anhydrous ethylamine (0°C, 4 h, under N_2); III_{II} was prepared by treatment of II with supercritical ammonia (135°C, 136 atm, 1 h); IV_I was prepared from III_I by heating in dry glycerol (260°C, 0.5 h, under N_2) followed by filtration and sequential washing with dimethylformamide and methanol. Amorphous (decrystallized) cellulose was also prepared by paraformaldehyde / dimethylsulfoxide / sodium methoxide treatment, or by ball-milling. Powder x-ray diffraction was used to verify that each conversion proceeded essentially to completion. Acid hydrolysis kinetics in 6 *N* HCl was used to measure degree of crystallinity. Particle size was measured by light microscopy, and gross surface area estimated by applying particle size data to geometric mensuration formulas derived for idealized, smooth cylindrical rods or rectangular parallelpipeds.

The in vitro digestion kinetics of the substrates were determined using pure cultures of the ruminal cellulolytic bacteria *Ruminococcus flavefaciens* FD-1 or *Fibrobacter succinogenes* S85 (grown in cellulose I - limited chemostats at D = 0.07 h^{-1}), or using mixed rumen microflora from two different donor cows. Fermentations were conducted under a CO_2 gas phase in 50 ml serum vials containing 100 mg of cellulose, 8 ml of medium (Modified Dehority's medium for pure cultures, N-supplemented McDougall buffer for mixed rumen microflora), 0.15 ml of cysteine-based reducing agent, and 1.8 ml inoculum. For each substrate, paired vials were sacrificed at 6-8 time points during the period of active fermentation (10-40 h), and the cellulose recovered on glass fiber filters by a modified neutral detergent fiber procedure. Weight loss data were fitted to a discontinuous first-order kinetic equation to yield a rate constant and a discrete lag time.

Results and Discussion

The chemical methods used to prepare the alternate allomorphs (II, III_I, III_{II}, IV_I) caused only slight changes in fiber dimensions, degree of crystallinity, and gross specific surface area from that of the original starting material (I). The amorphous cellulose had a crystalline content of <10%, but also had a much smaller particle dimensions and a fourfold greater gross specific area. All of the allomorphs retained their specific crystalline structure in control incubations in culture media in the absence of microbial inoculum, except for III_I, which showed a slight tendency to revert to I.

Digestion kinetics data are shown in Table 1. The different inocula displayed kinetic profiles which differed considerably from one another. The first-order rate constants for degradation of the substrates by pure cultures followed the order: Amorphous > IV_I > III_I > III_{II} > I > II; in fact, *R. flavefaciens* did not degrade II even after an incubation of several weeks. While the mixed rumen microflora from both donor cows degraded amorphous cellulose and I at similar rates and degraded II and III_{II} relatively slowly, they varied considerably with respect the degradation rate of III_I and IV_I.

Interpretation of the pure culture data on the basis of the proposed cellulose structural models of Sarko suggest the following: 1) Intersheet hydrogen bonding (and possibly antiparallel chain packing), as is thought to occur in celluloses II and III_{II}, have a strong negative effect on the degradation of the fibers; 2) Rotation of the O(6) hydroxyl position of the glucosyl residues (which is the chief distinguishing feature of IV_I) enhance the degradation rate; and 3) Skewing of adjacent sheets with respect to one another (as occurs in cellulose III_I and III_{II}) enhances the degra-

dation rate either by yielding a less thermodynamically stable unit cell or by exposing more of the chain ends to cellulolytic enzymes.

The similarity in degradation rate for I and amorphous cellulose reinforce our previous work showing that degree of crystallinity is not an important determinant of cellulose degradation by the bulk rumen population (although the lag period prior to the onset of degradation appears to be shorter for amorphous cellulose than for the crystalline allomorphs). Several explanations may be advanced for the disparity between the pure culture and mixed microflora data. The strains of *R. flavefaciens* FD-1 and *F. succinogenes* S85, while the benchmark strains for laboratory studies of ruminal cellulolytic bacteria, may not be representative of the strains or species found in the rumen. Alternatively, these organisms may represent the ruminal cellulolytic bacteria, but might show different substrate preferences when in the presence of the many other microbial species in the complex rumen environment.

Table 1. First-order kinetics for the degradation of cellulose allomorphs by pure cultures of ruminal cellulolytic bacteria and by mixed rumen microflora.

Allomorph	*R. flavefaciens*	*F. succinogenes*	Cow 507-J	Cow 748
I	1.00 ± 0.13	1.00 ± 0.06	1.00 ± 0.06	1.00 ± 0.05
II	—0—	0.32 ± 0.06	0.27 ± 0.01	0.71 ± 0.09
III_I	4.77 ± 0.06	1.75 ± 0.17	0.73 ± 0.02	1.56 ± 0.01
III_{II}	1.23 ± 0.19	1.34 ± 0.17	0.49 ± 0.01	0.82 ± 0.10
IV_I	4.08 ± 0.71	2.20 ± 0.49	0.89 ± 0.03	2.02 ± 0.07
Amorphous	9.58 ± 0.60	3.59 ± 0.39	0.84 ± 0.02	0.94 ± 0.01

THE EFFECT OF IONOPHORES AND PH ON THE GROWTH OF *STREPTOCOCCUS BOVIS* IN BATCH AND CONTINUOUS CULTURE

J. M. CHOW and J.B. RUSSELL

Introduction

Monensin and lasalocid are polyether antibiotics that inhibit the growth of Gram-positive bacteria, and these ionophores are routinely fed to feedlot cattle which consume large quantities of cereal grain. The benefit of ionophores has usually been ascribed to reductions methane formation, ammonia production, and coccidia, but they may also have a role in the prevention of ruminal acidosis. Ruminal acidosis causes depressions in food intake, tissue damage, founder, hemoconcentration, and in severe cases even death. *Streptococcus bovis* is a rapidly growing, Gram-positive ruminal bacterium that can produce large amounts of lactate and is involved in the onset of ruminal acidosis.

The effects of ionophores on pure cultures of ruminal bacteria have been examined in batch culture, but few experiments have been performed in continuous culture. Although the ruminal pH of feedlot cattle and high producing dairy cows can be less than 6.0, *in vitro* incubations have most often been preformed at near neutral pH. Because monensin and lasalocid are carboxylic ionophores, it seemed likely that pH would affect their antimicrobial activity.

Materials and Methods

Streptococcus bovis JB1 and S1 were grown anaerobically at 39°C in media containing salts, Trypticase, yeast extract, cysteine hydrochloride, glucose and 0 to 10 uM monensin or lasalocid. Continuous cultures were grown pH 6.7 or 5.7 at a dilution rate of 0.1 h^{-1} and the ionophore concentration was increased in a stepwise fashion. Optical density, glucose consumption, fermentation products and microbial protein were measured at each steady state. Washed cells were incubated with 0 to 10 uM ^{14}C-labeled monensin or lasalocid for 4 h at 39°C. Cells were harvested by centrifugation and the supernatant was removed. Pellets were resuspended in H_2O and ionophore binding was measured by liquid scintillation.

Results and Discussion

Batch cultures (pH 6.7) of *Streptococcus bovis* JB1 were severely inhibited by 1.25 and 5 uM lasalocid and monensin, respectively, even though large amounts of glucose remained in the medium. However, continuous cultures tolerated as much as 10 and 20 uM respectively and used virtually all of the glucose. Although continuous cultures grew with high concentrations of ionophore, the yield of bacterial protein decreased approximately 10-fold. When pH was decreased from 6.7 to 5.7, the potency of both ionophores increased, but lasalocid always caused a larger decrease in yield. The increased activity of lasalocid at pH 5.7 could largely be explained by an increased binding of the ionophore to the cell membrane. Because monensin did not show an increased binding at low pH, some other factor (e.g. ion turnover) must have been influencing its activity. There was a linear increase in lasalocid binding as the concentrations increased, but monensin binding increased markedly at high concentrations. Based on the observations that: 1) *S. bovis* cells bound significant amounts of ionophore (the ratio of ionophore to cell material was more important than the absolute concentration), 2) batch cultures responded differently than continuous cultures, and 3) pH can have a marked effect on ionophore activity, it appears that the term "minimum inhibitory concentration" may not provide an accurate assessment of microbial growth inhibition *in vivo*.

Based on a daily intake of 350 mg ionophore per day and a rumen volume of 70 liters, such a concentration seems realistic. However, *in vitro* incubations of ruminal bacteria contain much lower cell densities than those found *in vivo*. Assuming that bacterial protein concentrations *in vivo* are approximately 10 g/liter, a binding capacity of even 0.7 nmol/mg protein could completely remove all of the available ionophore (7uM). *S. bovis* (160 mg/liter) was able to bind as much 22 nmol monensin and 15 lasalocid per mg protein. It is not known it other rumen bacteria (e.g. Gram-negative bacteria) have as great a binding capacity as *S. bovis*, but it is likely that free ionophore concentrations *in vivo* are significantly less than 7 uM.

SODIUM-DEPENDENT TRANSPORT OF BRANCHED-CHAIN AMINO ACIDS BY A MONENSIN-SENSITIVE RUMINAL PEPTOSTREPTOCOCCUS

G. CHEN and J.B. RUSSELL

Introduction

For many years it had been assumed that most, if not all, of the predominant bacteria had been isolated from the rumen, but these species could not account for *in vivo* rates of amino acid degradation. Recently isolated ammonia-producing ruminal bacteria grew rapidly with amino acids or peptides as their sole energy source and

were present at significant numbers *in vivo*. One of them, a peptostreptococcus, produced large amounts of branched-chain volatile fatty acids, and these acids arose from the fermentation of branched-chain amino acids.

Monensin is often used as a feed-additive in beef cattle and dairy heifers, and it inhibits amino acid deamination. The reduction in wasteful amino acid fermentation is desirable, but monensin can also decrease branched-chain volatile fatty acid production. These acids are required by ruminal cellulolytic bacteria, and a deficiency can inhibit cellulose digestion. Because branched-chain volatile fatty acids can have a significant effect on feed digestion, we decided to examine the metabolism of branched-chain amino acids by the ruminal peptostreptococcus in greater detail.

Material and Methods

The ruminal peptostreptococcus was grown anaerobically in medium containing salts, cysteine hydrochloride, Casaminoacids, vitamins and microminerals (pH 6.7). Sodium deficient medium contained only potassium salts and purified amino acids. The uptake of ^{14}C amino acids was monitored in whole cells or membrane vesicles. An artificial membrane potential or chemical gradient of sodium was created by valinomycin treatment and potassium diffusion. Intracellular pH was measured by method employing ^{14}C benzoin acid and the membrane potential was estimated from the uptake of ^{14}C tetraphenylphosphonium. Intercellular sodium was measured by atomic absorption and ATP by luminescence.

Results and Discussion

The ruminal peptostreptococcus which produced large amounts of branched-chain volatile fatty acids grew rapidly with leucine as an energy source in the presence but not the absence of sodium. Leucine transport could be driven by an artificial membrane potential only when sodium was available, and a chemical gradient of sodium also drove uptake. Because sodium was taken up with leucine and a pH gradient could not serve as a driving force (with or without sodium), it appeared that leucine was transported in symport with sodium. The leucine carrier could use lithium as well as sodium and had a single binding site for sodium. The affinity constant for sodium was 5.2 mM, and affinity constant and maximum velocity for leucine were 77 uM and 328 nmol/mg protein/min, respectively. Since valine and isoleucine competitively inhibited leucine transport, it appeared that the peptostreptococcus used a common carrier for branched-chain amino acids. Valine or isoleucine were taken up rapidly, but little ammonia was produced it they were provided individually. The lack of ammonia could be explained by an accumulation of reducing equivalent inside the cell. The ionophore, monensin, inhibited growth, but leucine was taken up and deaminated at a slow rate. Monensin caused a loss of potassium, an increase in sodium, a slight increase in membrane potential and a decrease in intracellular pH. The inhibition of growth was consistent with a large decrease in ATP. The capacity of the ruminal peptostreptococcus to transport and deaminate branched-chain amino acids and its sensitivity to monensin clarifies the observation that monensin decreases branched-chain volatile fatty acid production in vivo.

Monensin is an ionophore which is primarily effective against Gram-positive bacteria. For many years it was assumed that *Megasphaera elsdenii* was the primary producer of ruminal branched volatile fatty acids, but its resistance to monensin could not explain the negative relationship between monensin and decreased branched-chain volatile fatty acids. The peptostreptococcus was sensitive to monensin, and this sensitivity may explain the observation that exogenous branched-chain volatile fatty acids overcame the negative effect of monensin on milk production.

CLONING AND SEQUENCING OF A *BACTEROIDES RUMINICOLA* ENDOGLUCANASE GENE

O. MATSUSHITA, J.B. RUSSELL and D.B. WILSON

Introduction

Ruminant animals depend on microorganisms to digest cellulose, but only a few species of rumen bacteria can digest cellulose. In modern feeding situations, cereal grains are often added to ruminant diets because starch is fermented faster than fibrous materials. Starch fermentation can induce an overgrowth of lactic acid-producing bacteria, with a subsequent lowering of ruminal pH. The ruminal pH of feed-lot cattle and high-producing dairy cattle is often less than 6.0. Cellulolytic rumen bacteria are sensitive to even small declines in pH, and little ruminal cellulose digestion occurs when the pH is less than 6.0. *Bacteroides ruminicola is* a noncellulolytic that is often found at high numbers in the rumen. It is able to grow at pH 5.1 and is able to utilize the products of cellulose digestion. Preliminary experiments indicated that *B. ruminicola* produced a carboxymethylcellular (CMCase) even though it was unable to grow on ball-milled or acid-swollen cellulose. Given these properties, it seemed possible that *B. ruminicola* might be genetically modified to produce an acid-tolerant, cellulolytic rumen bacterium. To study the CMCase and its regulation more completely, we cloned the *B. ruminicola* CMCase gene into E. coli.

Materials and Methods

B. ruminicola DNA was digested with the restriction enzyme Eco R1 and the DNA fragments were ligated into lambda phage. E. coli was infected with the lambda phage and CMCase plaques were screened by a Congo Red overlay procedure. The Eco R1 fragment of DNA from positive plaques was digested with BAMHI and Sphl. A Hind III fragment was then subcloned into pUC18 in both directions relative to a lac promoter and each construct was tested for inducibility by IPTG. Nested deletions were then sequenced by the method of Sanger. The N-terminal amino acid sequence was determined with a model 470A sequence (Applied Biosystems). The CMCase was purified, anitbodies were raised against the protein, and Western blots performed.

Results and Discussion

B. ruminicola $B_1 4$ produced and endoglucanase (CMCase) that was excreted into the culture supernatant. Cultures grown on glucose, fructose, maltose, mannose and cellobiose had high specific activities of CMCase, but its synthesis was repressed by sucrose. *B. ruminicola* did not grow on either ball-milled or acid-swollen cellulose even the CMCase could hydrolyze acid-swollen cellulose. The CMCase lacked a binding site for cellulose. The CMCase gene was cloned into *E. coli,* unidirectional deletion analysis indicated that it was a Hind III, 1.16 kb fragment. Its nucleotide sequence contained a single open reading frame coding a protein of 40,481 daltons. The enzyme was overproduced in *E. coli* under the control of a tac promoter and purified homogeneity. The N-terminal sequence, amino acid composition, and molecular weight of the purified enzyme were similar to values predicted from the open reading frame of the DNA sequence. However, the CMCase present in *B. ruminicola* was found to have a monomer molecular weight of 88,000 by Western immunoblotting. This discrepancy appeared to have resulted from our having cloned only part of the CMCase gene in *E. coli.*

Antiserum raised against the 40,500 molecular weight CMCase isolated the *E. coli* carrying the *B. ruminicola* CMCase gene reacted with two *B. ruminicola* endoglucanases which had molecular weights of 82,000 and 88,000. The *B. ruminicola* enzymes were purified and their N-terminal amino acid sequences were determined. DNA sequence encoding these enzymes was determined both from cloned fragments and from a product of *B. ruminicola* chromosomal DNA. All the sequences were identical. The 88,000 CACase appears to be produced from two open reading frames which overlap for 18 base pairs. The sum of the predicted molecular weights of the two open reading frames is 106,000 which is significantly larger than the 88,000 molecular weight determined for the pure protein. An unusual event, such as ribosomal hopping, may be involved in translating the 88,000 CMCase in *B. ruminicola*. The entire gene was reconstructed in *E. coli* from two clones each carrying a part of the gene to examine if full-sized CMCase was produced in *E. coli*. The entire gene produced CMCase of 88,000, 84,000 and 40,500. We are currently attempting to add a cellulose binding site coding region to the CMCase. If the reconstructed gene is active against crystalline cellulose it will be returned to *B. ruminicola*.

GENETIC VARIATION IN ALFALFA FOR RUMINAL PROTEIN DEGRADABILITY

G. A. BRODERICK and D. R. BUXTON

Introduction

Protein in alfalfa forages is extensively degraded in the rumen, and, although degraded protein can be reincorporated into protein by ruminal microbes, forage proteins are often wasted due to excessive ammonia formation in the rumen. Results from feeding studies have confirmed that alfalfa protein may be utilized inefficiently by lactating dairy cows. Presence of condensed tannins in some legume species decreases ruminal protein degradation, either by altering the forage proteins and/or by inhibiting microbial proteases. Alfalfa does not have detectable quantities of tannins in its herbage. However, protein in legumes not containing tannins varies in susceptibility to proteolytic attack. Albrecht and Muck (Crop Sci. 31:in press, 1991) found that red clover silage contained significantly less nonprotein nitrogen than silage made from birdsfoot trefoil, which contains tannins, and alfalfa which does not. The objective of this study was to determine if there are potential genetic differences in the ruminal degradability of alfalfa forage protein.

Materials and Methods

Twenty-two entries of alfalfa germplasm, including 19 of *Medicago sativa* [two commercial cultivars (Agate and Saranac AR) and 17 plant introductions (PI)], plus three PI of *M. falcata,* were collected during the 1984 growing season in Ames, IA. Duplicate samples were taken when forage was harvested between 25 to 27 June and 20 to 22 Aug (total 88 samples). Each sample of about 300 g herbage was dried at 55°C and ground to pass a 1-mm screen of a cyclone mill. Samples were analyzed for DM, total N (TN), ADF and ADIN. Rates of ruminal protein degradation (k_d), and fractions degraded at 0-h (fraction A) and potentially degradable (fraction B) were determined using the inhibitor in vitro system described earlier (Broderick, Brit. J. Nutr. 58:463, 1987); each set of incubations was repeated four times. Casein was included as a standard protein in each incubation. Degradation rates were corrected for unavailable N (fraction C), based on ADIN content. Net extents of ruminal protein escape (i.e., corrected for ADIN) were computed using the equation:

Estimated Protein Escape, % = $B[k_p/(k_d + k_p)]$

where k_p, the ruminal passage rate, was assumed equal to be .06 /h.

Results and Discussion

Alfalfa ADF, TN, and ADIN (Table 1) ranged from 35.8 to 41.2% of DM, 2.38 to 2.77% of DM, and 5.96 to 7.34% of TN, respectively; there were no significant differences in ADF ($P = .64$), TN ($P = .22$), or ADIN ($P = .31$) among alfalfa entries. However, ADF was significantly greater, and TN significantly lower, in first than second harvest ($P < .001$). Also, there was a significant harvest x replicate interaction for ADIN ($P < .05$): ADIN was similar between replicates during Harvest 1, averaging 6.76% of TN, but averaged 6.21 and 6.87% of TN for Replicates 1 and 2 during Harvest 2. Significant effects of germplasm source were detected for the four parameters determined by using the ruminal IIV system (Table 1): fraction A (degraded N at 0-h) ($P < .002$), fraction B ($P < .0025$), degradation rate (k_d) ($P < .001$), and net protein escape ($P < .001$). Because harvest and replicate were

confounded by in vitro incubation date, it was not possible to test their effects on the degradation parameters.

With few exceptions, *M. sativa* had larger fractions A and smaller fractions B than the three entries of *M. falcata* (Table 1). Three of the *M. sativa* entries, PI 171721, 198963, and 215671, were not different from *M. falcata* for both fractions. Similarly, the 19 entries of *M. sativa* had more rapid degradation rates (k_d), ranging from .21 to .25/h, whereas those for *M. falcata* averaged .18/h (Table 1). Estimated net protein escapes from the rumen averaged 17.1 and 21.2% for *M. sativa* and *M. falcata*, respectively. Consistently larger fractions B and slower degradation rates yielded estimated net protein escapes for the three *M. falcata* entries which were greater than 18 of 19 *M. sativa* entries (Table 1). These differences did not seem to be due to variation in N content because *M. sativa* and *M. falcata* averaged 2.52 and 2.53% TN. The mean ruminal protein escape of 21.2% for *M. falcata* is not adequate for optimal protein utilization in lactating cows or in other productive ruminants, but this value represented a 24% relative increase over the mean net protein escape of 17.1% observed for *M. sativa*. The 1988 Dairy NRC reported ruminal protein escapes for alfalfa hay and silage of 28 and 23%; these values may be compared to estimated total escapes (i.e., including fraction C) for *M. sativa* and *M. falcata* of 22 and 27%.

Degradation rates obtained for casein and the 22 entries of alfalfa, graphed from highest to lowest, are in Figure 1. This graph illustrates the consistently slower degradation rates for the three *M. falcata* entries (PI numbers 258750, 262532, and 315476). Also shown in Figure 1 is the spread of differences among the *M. sativa* entries. Significant differences were observed among *M. sativa* entries for degradation rates and net protein escape (Table 1). This indicates that selection among or within accessions of *M. sativa* could effect genetic shifts for these characters.

Summary and Conclusion

Genetic differences were found in the ruminal degradability of protein in the alfalfa species *M. sativa* and *M. falcata*. Although estimated ruminal protein escape in *M. falcata* was 24% greater than *M. sativa*, significant differences in degradation rate also were detected among accessions of *M. falcata*. These results suggest that conventional plant breeding techniques may be used to develop lines of alfalfa with reduced ruminal degradation and increased protein utilization.

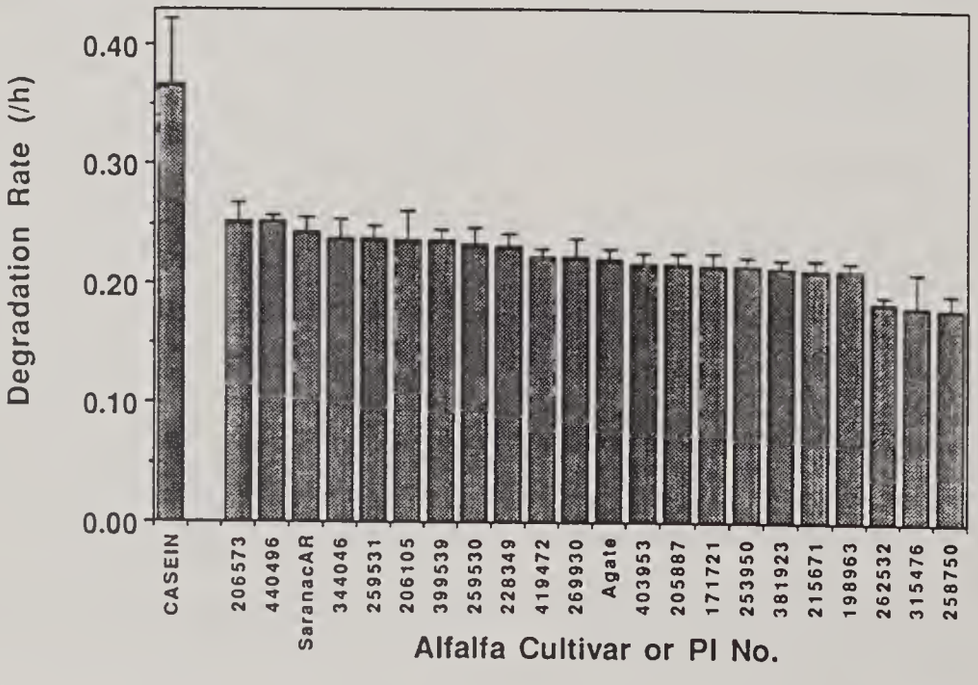


Figure 1. Fractional rates of ruminal protein degradation in 22 entries of alfalfa germplasm.

Table 1. Summary of composition and ruminal in vitro degradation data for samples of alfalfa germplasm.

Protein, cultivar or PI No.	*Medicago* species	Origin	ADF	TN	A	B	ADIN	k_d	Net protein escape
			(% DM)		----------(% TN)----------			(/h)	(%)
Casein	--------	--------	-----	13.74	.1	99.9	------	.366	15.2
Agate	*sativa*	USA	36.9	2.67	14.7^{abcde}	78.9^{bcd}	6.45	$.220^{cd}$	17.1^{cde}
SaranacAR	*sativa*	USA	37.8	2.38	14.6^{abcde}	78.6^{bcd}	6.77	$.242^{abc}$	16.1^{cde}
171721	*sativa*	Turkey	38.3	2.55	13.0^{defg}	79.9^{abc}	7.11	$.214^{cde}$	17.7^{c}
198963	*sativa*	Cyprus	38.1	2.40	12.6^{efg}	80.6^{ab}	6.81	$.210^{def}$	18.1^{bc}
205887	*sativa*	France	39.3	2.54	14.2^{abcde}	79.3^{bcd}	6.49	$.216^{cd}$	17.5^{cde}
206105	*sativa*	UK	38.6	2.39	15.5^{abcd}	77.6^{cd}	6.90	$.236^{abcd}$	16.3^{cde}
206573	*sativa*	Greece	39.3	2.66	16.7^{a}	76.8^{d}	6.51	$.252^{a}$	15.0^{e}
215671	*sativa*	Sweden	36.4	2.47	13.6^{cdef}	79.9^{abc}	6.52	$.211^{def}$	17.9^{c}
228349	*sativa*	Iran	38.4	2.61	14.5^{abcde}	79.0^{bcd}	6.57	$.230^{abcd}$	16.6^{cde}
253950	*sativa*	Afganistan	38.9	2.39	13.1^{defg}	79.7^{bcd}	7.29	$.214^{cde}$	17.7^{c}
259530	*sativa*	Bulgaria	41.2	2.52	13.8^{bcdef}	79.0^{bcd}	7.14	$.232^{abcd}$	16.4^{cde}
259531	*sativa*	Bulgaria	37.1	2.61	14.8^{abcde}	79.0^{bcd}	6.17	$.238^{abcd}$	16.2^{cde}
269930	*sativa*	Pakistan	38.2	2.44	13.9^{bcdef}	78.8^{bcd}	7.34	$.221^{bcd}$	17.2^{cde}
344046	*sativa*	Spain	36.1	2.67	14.9^{abcde}	79.1^{bcd}	5.98	$.238^{abcd}$	16.3^{cde}
381923	*sativa*	Canada	36.3	2.43	14.1^{abcdef}	79.1^{bcd}	6.85	$.213^{cde}$	17.6^{cd}
399539	*sativa*	Ethiopia	41.1	2.41	15.9^{abc}	77.1^{cd}	6.97	$.236^{abcd}$	15.9^{cde}
403953	*sativa*	USSR	38.2	2.51	13.0^{defg}	79.9^{abc}	7.12	$.216^{cd}$	17.5^{cde}
419472	*sativa*	Japan	37.5	2.52	14.0^{abcdef}	79.6^{bcd}	6.41	$.221^{bcd}$	17.2^{cde}
440496	*sativa*	USSR	37.8	2.77	16.6^{ab}	77.2^{cd}	6.25	$.251^{ab}$	15.2^{de}
258750	*falcata*	USSR	37.1	2.47	10.7^{g}	82.6^{a}	6.62	$.179^{g}$	21.1^{a}
262532	*falcata*	Israel	37.9	2.62	11.4^{fg}	82.6^{a}	6.00	$.184^{efg}$	20.5^{ab}
315476	*falcata*	USSR	35.8	2.50	11.3^{fg}	82.7^{a}	5.96	$.181^{fg}$	21.9^{af}

^{abcdefg}Means within columns for alfalfa germplasm with different superscripts differ ($P < .05$).

PROTEOLYSIS IN ENSILED FORAGE LEGUMES OF VARYING TANNIN CONCENTRATION

K.A. ALBRECHT and R.E. MUCK

Introduction

The breakdown of protein to soluble non-protein nitrogen during ensiling is a factor that significantly reduces the quality of alfalfa silage as a feed for ruminants. A class of compounds found in many legumes and known to bind proteins are tannins. This study sought to determine if proteolysis in forage legume silages was related to tannin concentration.

Methods

A variety of legume forages (alfalfa, red clover, sainfoin, cicer milkvetch, 4 sericea lespedeza cultivars, 4 birdsfoot trefoil cultivars) were established in 1987. In 1988, 2 additional cultivars each of sericea lespedeza and sainfoin, 2 alternate birdsfoot trefoil cultivars and *Lotus pedunculatus* were included in the study. The legumes were established in a randomized complete block design with four replications at Arlington, WI.

On 5 September 1987 and 31 August 1988, 1 kg of herbage was harvested from each plot. The forage was chopped by hand, and 50 g samples were sprayed with a silage inoculant (6.6×10^5 CFU/g forage) and ensiled in 100 ml centrifuge tubes. None of the forage was wilted, and two silos were ensiled per plot. After 35 days incubation at 30°C, the silos were opened for analysis.

Both the fresh and ensiled forage were analyzed for total Kjeldahl nitrogen, soluble non-protein N (NPN), ammonia, free amino acids, pH and moisture content. The fresh forage was also analyzed for tannin content.

Results

Tannin concentrations ranged from 0 to 27 and 0 to 31 g tannic acid equivalents/kg DM in 1987 and 1988 respectively. The pH and concentrations of total nitrogen, soluble NPN, ammonia, and free amino acids in the fresh and ensiled forages were within the range of previously published reports for forage legumes. With few exceptions, all silages were well preserved and showed no signs of clostridial fermentation.

The proportion of total nitrogen in the form of soluble NPN after ensiling ranged from 32 to 73% in 1978 and 26 to 69% in 1988 and was negatively correlated to tannin concentration ($r^2 = 0.75$ in each year) (Figure 1). Within sericea lespedeza, the only species with substantial variation in tannin content, coefficients of determination between soluble NPN and tannin were 0.81 in 1987 and 0.88 in 1988.

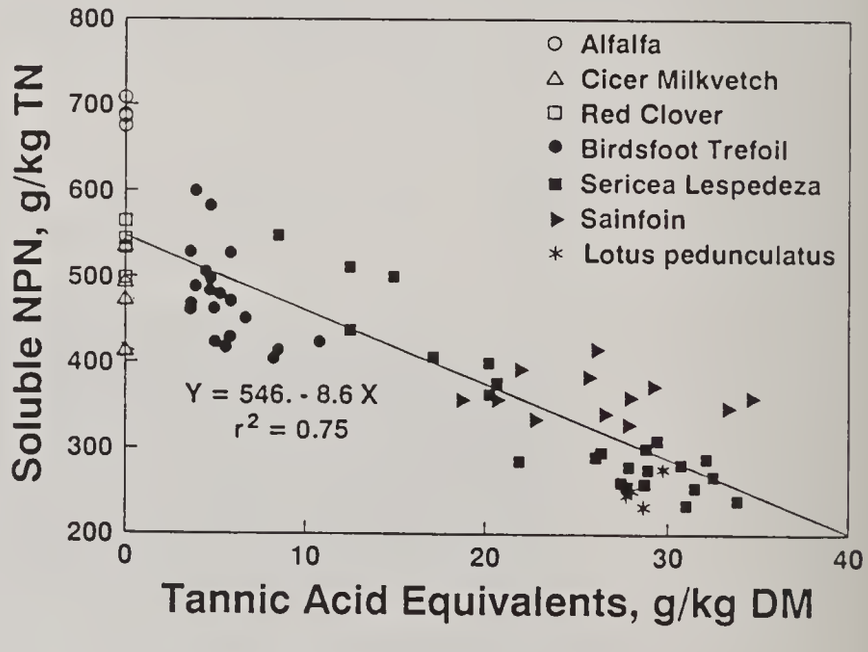


Figure 1. Relationship between fresh forage tannin concentration and soluble non-protein nitrogen in ensiled forage (1988).

Red clover and cicer milkvetch did not contain measurable levels of tannins but exhibited lower proteolysis than alfalfa, suggesting that other factors are involved in protein protection in these species.

Conclusions

Tannins apparently play a major role in limiting proteolysis in legumes during ensiling. However, other factors are also involved.

RESPONSE TO POSTRUMINAL INFUSION OF GLUCOSE OR PROTEIN DURING EARLY LACTATION IN DAIRY COWS FED ALFALFA SILAGE

T.R. DHIMAN and L.D. SATTER

Introduction

When high quality alfalfa silage constitutes the major portion of the dairy diet during early lactation, cows produce less milk with lower protein content. The objective of this study was to determine the limiting nutrient(s) for milk and milk protein production during early lactation in dairy cows fed diets based on alfalfa silage.

Materials and Methods

Twenty multiparous Holstein cows were randomly assigned to 4 treatments (5 cows in each treatment) at parturition. An adaptation period of 14 days was given during which all the cows were fed a pretrial diet containing forage:grain in the ratio of 48.2:50. On day 15 of experiment, cows in all treatments were changed to the experimental diet containing 98.2 percent forage. Treatment 1 was the control group whereas the cows in treatment 2, 3 and 4 were abomasally infused through the rumen cannula for 4 weeks with glucose (1 kg/d), soyprotein (1.22 kg/d) or glucose+protein (glu+prot) together. The ingredient composition of the diets is in Table 1. Daily feed intake and milk yield were recorded. Body weights were taken once weekly after the morning milking. Weekly blood samples were analyzed for glucose, urea, β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Blood samples were collected for plasma amino acid and insulin determinations.

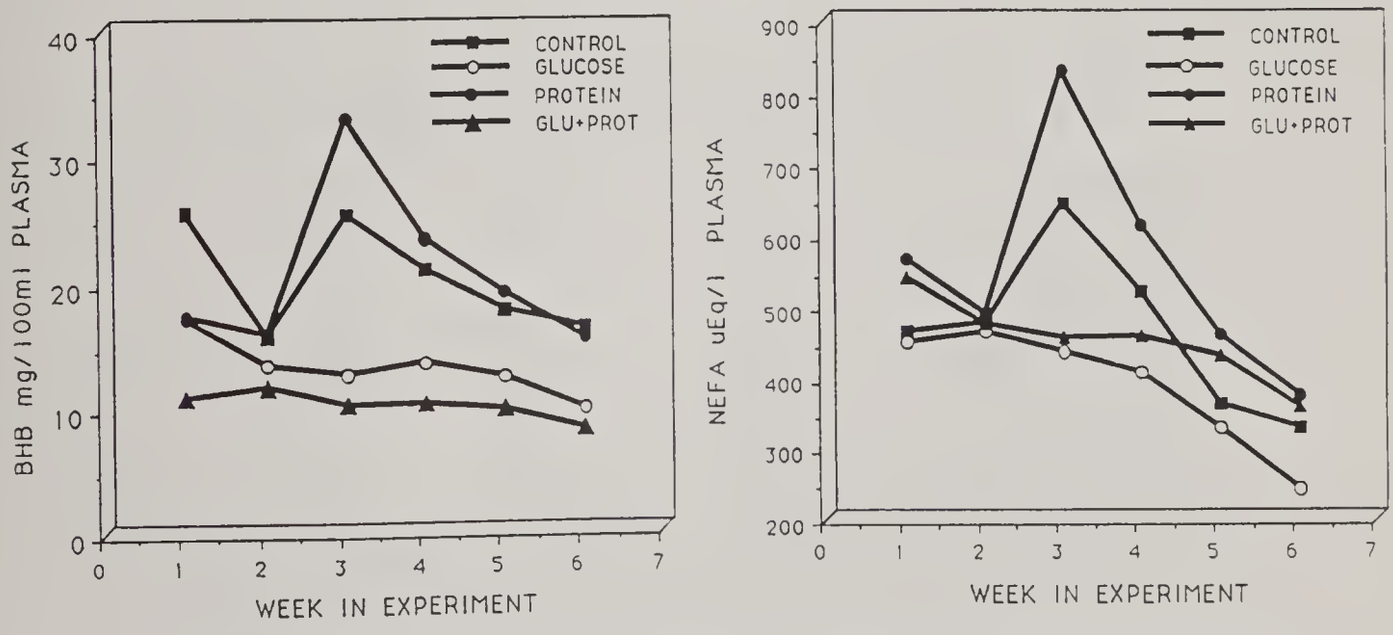


Figure 1. Effect of glucose or protein infusion on plasma β–hydroxybutyrate and non-esterified fatty acids.

Results and discussion

The responses to treatments are shown in Table 2. There was no change in DM intake due to protein or glu+prot infusion, but infusion of glucose did decrease daily DM intake compared to control. Daily milk and 3.5% FCM yield were significantly increased due to protein infusion compared to the control. There was no change in milk yield due to glucose infusion, but milk yield increased significantly when glucose was given along with protein. There was a slight increase in milk protein content due to protein infusion and further increase with glu+prot infusion. Glucose alone failed to increase milk protein content but was effective in reducing milk fat and protein content. The increase in milk yield due to protein or glu+prot was reflected in increased daily fat and protein yield. Infusion of glucose and glu+prot raised blood glucose concentration compared to protein infusion and the control. Immediately after the start of treatments, cows infused with protein had the highest levels of BHB and NEFA in blood plasma followed by control with nearly no change in cows infused with glucose or glu+prot (Figure 1), indicating, in early lactation, infusion of protein stimulates body tissue mobilization.

Conclusion

The response to protein infusion and lack of response to glucose infusion in early lactation cows fed high alfalfa silage diets suggests that, despite the fact that cows were deficient in both protein and energy, it was protein that was first limiting milk production with all forage diets.

Table 1. Ingredient composition of the experimental diets.

	Diet	
Ingredient	Pretrial	Experimental
Alfalfa silage	48.2	98.2
Shelled corn	34.0	- - -
Soybean meal	16.0	- - -
Dicalcium phosphate	1.1	1.1
Trace mineral and vitamin mix	0.7	0.7

Table 2. Effect of postruminal infusion of glucose or protein on dry matter intake, body weight change, milk and blood parameters.

	Treatment			
Parameter	Control	Glucose	Protein	Glu+prot
DM intake, kg/d	18.5^{a}	15.1^{b}	16.4^{ab}	17.7^{a}
Body weight change (wk6-wk2), kg	-19.0^{b}	-21.9^{b}	-28.2^{b}	12.2^{a}
Milk yield, kg/d	27.0^{c}	27.8^{bc}	30.5^{b}	33.9^{a}
3.5 % FCM, kg/d	26.7^{b}	25.3^{b}	31.2^{a}	32.8^{a}
Milk protein, %	2.79^{a}	2.52^{b}	2.84^{a}	2.95^{a}
Milk fat, %	3.61^{a}	2.98^{b}	3.61^{a}	3.32^{ab}
Glucose, mg/100 ml	55.6^{ab}	60.2^{a}	52.0^{b}	60.7^{a}
Insulin, uIU/ ml	5.1	5.6	6.0	6.4
Plasma BCAA, nM/ml	284^{ba}	244^{b}	389^{a}	238^{b}

[a,b,c]Values in the same row with different superscripts differ ($P<.05$).

RESPONSE TO INTRA-RUMINAL INFUSION OF PROPIONIC ACID OR ABOMASAL INFUSION OF PROTEIN DURING EARLY LACTATION IN DAIRY COWS FED ALFALFA SILAGE

T.R. DHIMAN and L.D. SATTER

Introduction

The objective of this study was to further examine the question of what nutrients limit milk and milk protein production during early lactation in dairy cows fed diets based on alfalfa silage.

Materials and Methods

Twenty multiparous Holstein cows at week 7 of lactation were assigned to 4 treatments (5 cows in each treatment). During an adaptation period of 14 days, all the cows were fed a pretrial diet containing forage:grain in the ratio of 48.2:50. On day 15 of the experiment, cows in all treatments were changed to the experimental diet containing 98.2 percent forage. The experimental period lasted until cows were 12 weeks into lactation. Treatment 1 served as the control group whereas the cows in treatments 2 and 3 were infused with propionic acid (750 g/d) into the rumen (Prop) and soyprotein (1.22 g/d) into the abomasum through the rumen cannula (Prot). Cows in treatment 4 were infused with propionic acid intra-ruminally and soyprotein into the abomasum (Prop+Prot). The ingredient composition of the diets is in Table 1. Daily feed intake and milk yield were recorded. Body weights were taken once weekly. Weekly blood samples were analyzed for glucose, urea, β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Blood samples for plasma amino acids and insulin assay were collected during week 11 of lactation.

Results and Discussion

The results are summarized in Table 2. Intra-ruminal infusion of propionic acid reduced DM intake by 29% ($P<0.05$) compared to the control. The effect of propionic acid in reducing DM intake was still evident when it was given along with protein into the abomasum. There was no change in DM intake due to protein infusion. Due to reduction in DM intake, cows lost the most body weight with

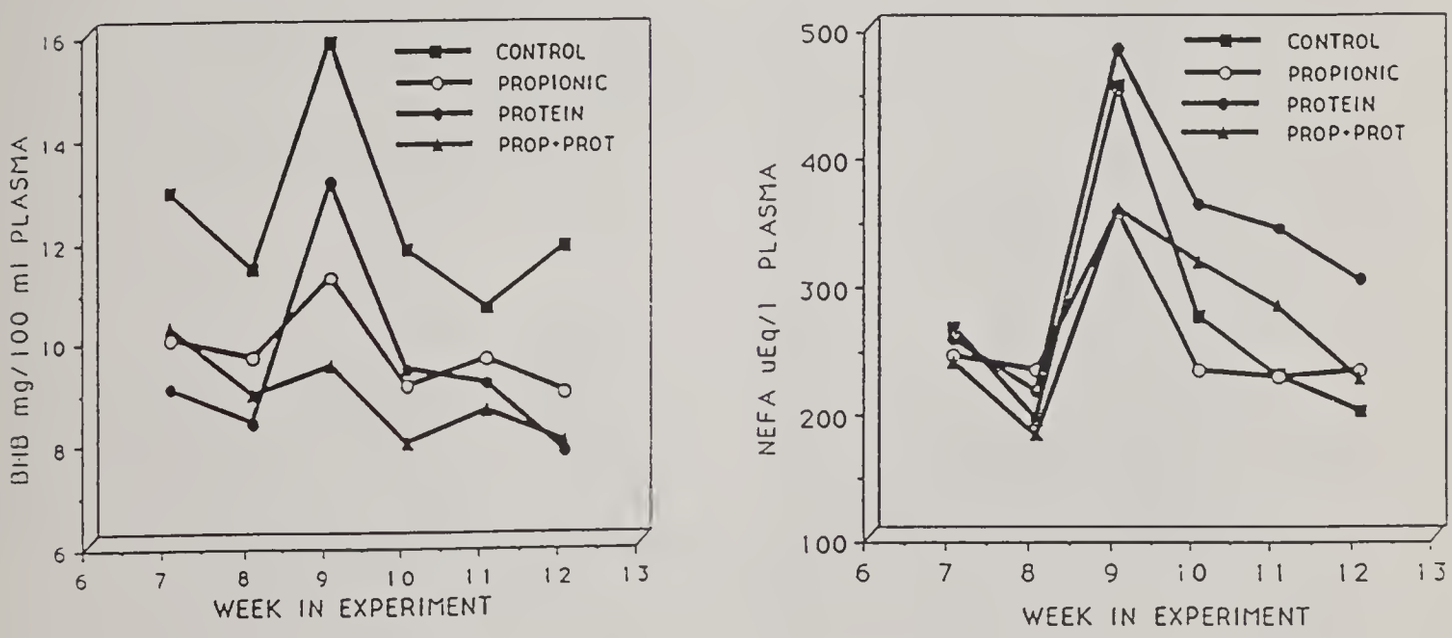


Figure 1. Effect of propionic acid and protein infusion on plasma β-hydroxybutyrate and non-esterified fatty acids.

propionic acid infusion followed by Prop+Prot and control. There was a significant increase in daily milk and 3.5 % FCM yield due to protein infusion compared to the control. In spite of reduced DM intake, the cows in the Prop+Prot infusion group produced about the same amount of milk as the control indicating that infusion of protein was compensating for the negative effect of propionic acid. Propionic acid and Prop+Prot infusion reduced milk protein and fat percent significantly. There was a slight increase in milk protein content due to protein infusion as compared to the control. Higher milk yield due to protein infusion was reflected in increased daily protein and fat yield. The cows in the control and protein infusion groups showed higher concentrations of plasma BHB and NEFA immediately after the start of treatment compared to propionic or Prop+Prot infusion cows (Figure 1).

Conclusion

The response to protein infusion in early lactation cows fed alfalfa silage diets in this experiment once again confirms that it was protein, not energy, that was first limiting milk production with all forage diets.

Table 1. Ingredient composition of the experimental diets.

Ingredient	Diets Pretrial	Experimental
Alfalfa silage	48.2.	98.2
Shelled corn	34.0	- -
Soybean meal	16.0	- -
Dicalcium phosphate	1.1	1.1
Trace mineral and vitamin mix	0.7	0.7

Table 2. Effect of intra-ruminal infusion of propionic acid and abomasal infusion of protein on DM intake, body weight change, milk and blood parameters.

Parameter	Treatment Control	Prop	Prot	Prop+Prot
DM intake, kg/d	19.9^{a}	14.1^{b}	18.3^{a}	13.4^{b}
Body weight change (wk12-wk8), kg	-1.0^{a}	-34.2^{b}	8.53^{a}	-10.3^{ab}
Milk yield, kg/d	23.0^{b}	19.8^{c}	26.8^{a}	24.5^{b}
3.5 % FCM, kg/d	22.9^{b}	18.4^{c}	26.6^{a}	23.3^{b}
Milk C protein, %	2.81^{a}	2.63^{b}	2.87^{a}	2.68^{b}
Milk fat, %	3.54^{a}	3.07^{b}	3.43^{ab}	3.14^{ab}
Glucose, mg/100 ml	60.2^{ab}	61.3^{ab}	59.0^{b}	63.3^{a}
Insulin, uIU/ ml	7.8	7.9	8.0	6.2
Plasma BCAA, nM/ml	247^{ab}	221^{b}	290^{ba}	338^{a}

^{a,b,c}Values in the same row with different superscripts differ (P<.05).

VALUE OF "RUMINANT-GRADE" FISH MEAL FOR LACTATING DAIRY COWS FED ALFALFA SILAGE AS SOLE FORAGE

G.A. BRODERICK

Introduction

Diets which appear to be adequate in crude protein (CP) can be formulated for dairy cows using only alfalfa forage plus feed grains with no protein concentrates. However, extensive degradation of alfalfa protein in the rumen results in inefficient utilization and depressed production of milk and milk protein. Moreover, extensive proteolysis during wilting and ensiling further impairs capture of alfalfa silage N in the form of milk. Thus, it is advantageous that cows fed alfalfa silage-based diets be supplemented with proteins which are resistant to rumen degradation.

We observed that fish meal was a significantly better protein source than soybean meal for lactating cows (Broderick, USDFRC 1988 Research Summaries, pp. 59-60, 1989). However, in vitro tests showed that estimated rumen escape of various fish meal proteins ranged from 43 to 72% (G. A. Broderick, unpublished); differences were largely due to variation in amounts of soluble protein added back during manufacture (S. Goldhor, personal communication). The purpose of the present trial was to determine if there was an advantage to supplementing fish meal with low levels of soluble protein to lactating cows fed alfalfa silage-based diets.

Materials and Methods

Thirty-two cows averaging 33 days in milk were fed diets containing one of the four protein supplements in a 4X4 Latin square (Table 1): Control (no supplement, C); soybean meal (SBM); High-Solubles Fish Meal (HSFM); and Low-Solubles Fish Meal (LSFM; "Sea-Lac" from Zapata-Haynie Corp., Hammond, LA). Extents of rumen protein degradation estimated in vitro for SBM, HSFM and LSFM were 27, 46 and 63%. Forage was from third-cutting alfalfa ensiled at 37% dry matter (DM; 20.6% CP and 43% NDF, DM basis) which comprised 56% of diet DM (Table 1). Each cow was fed its respective diet with the appropriate protein supplement for 3-week periods before switching to the next protein (total 12 weeks). Milk production data from week 1 were discarded, and the data analyzed were from the last 2 weeks of each period. Milk production was measured at each milking; milk samples were analyzed for fat, protein, lactose, SNF and urea. Cows were weighed on three consecutive days at the start of the trial and at the end of each period. Four hours after feeding on day 20 of each period, blood samples were taken from the tail vein of each cow and blood plasma was analyzed for glucose and urea.

Results and Discussion

Feed DM intake was unaffected by supplemental protein. Weight change was positive on LSFM and greater than on C; weight change on SBM or HSFM was not different from C (Table 2). Production of milk and all milk components was clearly greater with additional protein. Supplementation with any of the three proteins increased milk and 3.5% FCM an average 2.4 and 2.7 kg/day over diet C—increases of 7 and 8% (Table 2). Average secretion of fat, protein, lactose and SNF was increased by 10, 10, 6 and 8%, respectively, over C (Table 2). Milk fat content and production were not reduced by feeding either fish meal. Orthogonal contrasts revealed that feeding supplemental protein resulted in small but significant changes in milk content of protein and lactose ($P<.001$), and SNF ($P<.037$). Production of milk and SNF tended to be greater with the fish meals vs. the SBM diet: production was greater on LSFM than SBM, with production on HSFM being intermediate (Table 2).

The most unequivocal finding was increased protein secretion with addition of protein, particularly fish meal, to the diet. Milk protein content increased about .1% with the two fish meals compared to diets C and SBM (Table 2). Relative to C, protein secretion increased by 61, 95, and 130 g/day with addition of SBM, HSFM, and LSFM; these differences were all significant ($P<.05$; Table 2). Greater protein secretion with LSFM over HSFM indicated the advantage of feeding "ruminant-grade" fish meal which has reduced protein degradability. The increase in protein secretion with LSFM of 130 g/day was more than two-thirds of the response of 180 g protein/day obtained by Dhiman and Satter [J. Dairy Sci. 72 (suppl. 1): 299, 1989] with the infusion of casein directly into the abomasum of early lactation cows. Protein supplementation did not alter blood glucose but increased milk and blood urea. There were no differences among urea concentrations obtained with the different proteins.

Summary and Conclusion

Protein supplementation of an alfalfa silage basal diet containing over 16% CP resulted in increased production of milk and milk components. There was increased production of milk and most milk components with feeding of fish meal vs. SBM, the most common protein supplement fed to dairy cattle. Feeding 3.7% fish meal with a 56% alfalfa silage diet did not reduce milk fat content or production. Greater protein secretion with LSFM vs. HSFM is probably due to greater rumen protein escape. This indicates the importance of assuring that fish meal or other "high-bypass" protein supplements are actually resistant to rumen breakdown.

Table 1. Composition of diets.

Ingredient	Control	SBM	HSFM	LSFM
		(% of DM)		
Alfalfa Silage	56.00	56.06	56.07	56.07
High Moisture Corn	42.75	37.33	38.99	39.00
Soybean Meal		5.36		
High Solubles Fish Meal			3.69	
Low Solubles Fish Meal				3.68
DiCal	.67	.67	.67	.67
TMS (+ Se)	.48	.48	.48	.48
Vitamins ADE Conc.	.10	.10	.10	.10
Composition				
Crude Protein (%)	16.15	18.17	18.28	18.25
NE_l (Mcal/kg)	1.64	1.63	1.62	1.62
NDF (%)	29.4	29.5	29.3	29.3

Diets were fed as total mixed rations.

Table 2. Dry matter intake, body weight gain, and production of milk and milk components.

	Protein supplement				STATS
Item	Control	SBM	HSFM	LSFM	Prob. Protein effect
CP Supplement, g/day	0	558	539	542	- - -
DMI, kg/day	21.2	21.5	21.4	21.7	.517
BW gain, kg/day	-.50[b]	-.01[ab]	-.19[ab]	.18[a]	.006
Milk, kg/day	33.9[c]	35.7[b]	36.2[ab]	36.9[a]	<.001
3.5% FCM, kg/day	32.3[c]	34.5[b]	34.9[ab]	35.6[a]	<.001
Milk fat, %	3.23	3.32	3.33	3.31	.538
Milk fat, kg/day	1.087[b]	1.173[a]	1.188[a]	1.210[a]	<.001
Milk protein, %	2.81[b]	2.84[b]	2.90[a]	2.93[a]	<.001
Milk protein, kg/day	.949[d]	1.010[c]	1.044[b]	1.079[a]	<.001
Milk lactose, %	4.94[ab]	4.96[a]	4.90[bc]	4.87[c]	<.001
Milk lactose, kg/day	1.669[b]	1.765[a]	1.768[a]	1.792[a]	<.001
SNF, %	8.35[b]	8.41[ab]	8.43[a]	8.44[a]	.037
SNF, kg/day	2.822[c]	2.993[b]	3.040[ab]	3.104[a]	<.001

Control = no protein supplement; SBM = solvent soybean meal; HSFM = high solubles fish meal; LSFM = low solubles fish meal; DMI = dry matter intake; BW = body weight; FCM = fat-corrected milk.
[a,b,c,d]Means in rows with different letters differ (P<.05).

EFFECT OF FEEDING EARLY LACTATION MULTIPAROUS COWS HEAT TREATED FULL FAT SOYBEANS

M. T. SOCHA and L. D. SATTER

Introduction

Heat treatment has been demonstrated to increase the rumen undegraded protein fraction found in soybeans. The two major heat treatments used by industry today are extrusion and roasting. This study was conducted to determine the production response of early lactation cows fed either solvent soybean meal, raw soybeans, extruded soybeans or roasted soybeans with alfalfa silage as the sole forage source.

Materials and Procedures

The soybeans were roasted with a Gem Roaster (soybeans exiting the roaster were 150°C) and held at 130°C for 30 minutes in one of four 30 bushel compartments in a specially designed wagon. The roasted and raw soybeans were cracked before storing. The extruded soybeans used were the HTP product supplied by Triple F Feed Company, Des Moines, Iowa. Sixty multiparous cows were sequentially assigned to one of four rations on day 15 of lactation. The rations consisted of 50% alfalfa silage, 35 - 38% rolled shell corn, 2% vitamin-mineral mix and one of four protein supplements (10% SBM; 13% raw soybeans; 13% extruded soybeans; or 13% roasted soybeans) on a dry matter basis. The vitamin-mineral mix was added to obtain a 1% calcium level in the ration on a dry matter basis. Diets contained 17% crude protein and were fed as total mixed rations. The SBM diet was fed during the first 14 days of lactation. Days 8 to 14 of lactation were used for covariate adjustment of milk production and composition. Milk production and feed intake were measured daily through week 20 of lactation. Each week milk samples were taken

and analyzed for fat and protein composition. Body weights and body condition scores were also recorded weekly.

Results and Discussion

Dry matter intakes (Table 1) were lower for the cows on the raw and roasted soybean treatments. Cows on the extruded soybean diet produced more milk than cows on soybean meal and raw soybean treatments and produced more 3.5% FCM than cows on the raw soybean diet (Table 2). Milk protein production did not differ since cows on the roasted soybean and extruded soybean rations had lower milk protein percents. Body weight changes and body condition scores did not differ for cows on the four treatments.

Conclusion

Heat processing of soybeans can greatly increase their value as protein supplements for lactating cows that are in their second or subsequent lactation.

Table 1. Intakes and ration composition.

	Treatment			
Item	Soybean Meal	Heated Soybeans	Extruded Soybeans	Raw Soybeans
Intake				
DM, kg/d[1]	24.7^{A}	22.5^{B}	25.1^{A}	22.7^{B}
DM, % of BW	4.01^{AB}	3.83^{AB}	4.10^{A}	3.74^{B}
CP, kg/d	4.32	3.87	4.29	3. 88
UIP, kg/d	1.49^{C}	1.61^{B}	1.79^{A}	1.20^{D}
Ne_L, Mcal/d	38.3	35.6	39.7	35.9
Diet (Dry Basis)				
Crude Protein (%)	17.5	17.2	17.1	17.1
Ne_L, Mcal/kg[1]	1.55	1.58	1.58	1.58
UIP, %of total CP	34.0	42.0	42.0	31.0

^{A,B,C,D}Means in the same row with different superscripts differ P<.05.
[1]Estimated from NRC values.

Table 2. Production response and body weight and body score.

Item	Soybean Meal	Heated Soybeans	Extruded Soybeans	Raw Soybeans
Yield, kg/d				
Milk[1]	36.0^{a}	37.5^{ab}	39.0^{b}	35.9^{a}
FCM, 3.5%[1]	35.7^{ab}	36.6^{ab}	37.6^{a}	35.2^{b}
Protein	1.16	1.06	1.14	1.06
Milk Composition, %				
Fat[1]	3.48^{a}	3.35^{ab}	3.25^{b}	3.45^{a}
Protein[1]	3.10^{a}	2.95^{bc}	2.89^{c}	3.00^{ab}
Body Weight, kg				
Beginning	598	592	612	567
Ending	624	621	637	587
Body Condition				
Beginning	3.22	3.25	3.26	3.16
Ending	3.28	3.32	3.27	3.23

^{abc}Means in the same row with different superscripts differ P<.05.
[1]Treatment means were covaried on pretreatment means.

EFFECT OF FEEDING PRIMIPAROUS COWS ROASTED FULL FAT SOYBEANS

M. T. SOCHA and L. D. SATTER

Introduction

Heated full fat soybeans are an excellent source of fat and ruminal undegraded protein. However, research has shown a limited response by primiparous cows to the feeding of either fat or ruminal undegraded protein. The purpose of this study was to compare the response of primiparous cows to the feeding of either heated full fat soybeans or solvent soybean meal.

Materials and Procedures

The soybeans used in this study were heated with a Gem Roaster (soybeans exiting the roaster were 150°C) and held at 130°C for 30 minutes in one of four 30 bushel compartments in a specially designed wagon. Thirty-two primiparous cows were sequentially assigned to one of two rations containing either the roasted soybean or soybean meal as the protein source on day 15 of lactation. The rations contained 50% alfalfa silage, 35 or 38% rolled shell corn, 2% vitamin-mineral mix and either 13% heated soybeans or 10% soybean meal on a dry matter basis. The vitamin-mineral mix was added to obtain a 1% calcium level in the ration on a dry matter basis. Diets contained 17% crude protein and were fed as a total mixed ration. The SBM diet was fed for the first fourteen days of lactation. Days 8 to 14 of lactation were used for covariate adjustment of milk production and composition. Milk production and feed intake were recorded daily through week twenty of lactation. Milk samples were collected once weekly and analyzed for fat and protein composition. Body weights and body scores were recorded once weekly.

Results and Discussion

Dry matter intakes did not differ between the two treatments (Table 1). Cows on the soybean meal diet tended to have a higher crude protein intake while cows on the heated soybean treatment tended to have higher UIP intakes. There was no difference in Ne_L intake and no difference in milk production or 3.5% FCM production. Cows on the heated soybean treatment had higher fat percent while the cows on the soybean meal diet tended to have higher milk protein percent. However, there was no difference in milk protein production.

Conclusion

First lactation heifers did not respond with increased milk production to feeding of heat processed soybeans.

Table 1. Intakes and ration composition.

	Treatment	
Intake	Soybean Meal	Roasted Soybeans
DM, kg/d	18.7	18.2
Dm, % of BW	3.63	3.54
CP, kg/d	3.20	3.06
UIP, kg/d	1.09[B]	1.29[A]
Ne_L, Mcal/d	29.0	28.8
Diet (Dry Basis)		
% Crude Protein	17.1	16.8
Ne_L, Mcal/kg[1]	1.55	1.58
UIP, % of total CP	34.0	42.0

[AB]Means in the same row with different superscripts differ P<.05.

[1]Estimated from NRC values.

Table 2. Production response and body weight and body score.

Item	Soybean Meal	Heated Soybeans
Yield, kg/d		
Milk[1]	28.2	28.4
FCM, 3.5%[1]	27.5	28.3
Protein[1]	0.84	0.84
Milk Composition, %		
Fat	3.34	3.50
Protein[1]	3.02[A]	2.92[B]
Body Weight, kg		
Beginning	513	517
Ending	537	540
Body Condition		
Beginning	3.35	3.09
Ending	3.27	3.19

[AB]Means in the same row with different superscripts differ P<.05.
[1]Treatment means were covaried on pretreatment means.

EFFECT OF DIETARY FAT SOURCE ON MILK FATTY ACID COMPOSITION

T.R. DHIMAN, K. VAN ZANTEN AND L.D. SATTER

Introduction

The use of fats/oils in the dairy diet has increased greatly. They can affect milk fat as well as milk fatty acid content. The objective of this study was to compare the effect of different dietary fat sources on milk fatty acid composition.

Materials and Methods

Four multiparous cows fitted with rumen cannula and 4 intact primiparous cows in mid lactation were used in a 4x4 Latin square design. Each period was 21 days. The four treatments were control (CTL), roasted soybeans (RSB), megalac (MG) and alifat (AL). The ingredient composition of the diets is in Table 1. Diets were offered as total mixed rations. Milk samples were collected from two consecutive milkings (p.m. and a.m.) on days 4, 7, 11, 14, 18 and 21 of each period. The samples were analyzed for fat, protein and milk fatty acid composition. During the last 3 days of each period, bags containing 5 g (DM basis) of alfalfa silage, silage + megalac and silage + alifat were suspended in the rumen of cannulated cows to determine forage DM disappearance rates. Megalac and alifat were added to the bags in the same proportion as were present in the diet. Rumen liquor and blood samples were collected during the last day of each period.

Results and Discussion

Supplementation of fat in treatment 3 decreased DM intake by 6.4% in multiparous cows and 11.4% in primiparous cows. Cows fed supplemental fat through RSB, MG or AL produced an average of 2.4 kg more 3.5% FCM and 88 g more milk fat daily. Milk fat percent was increased due to supplementation of megalac compared to the control. The milk protein percent was decreased with addition of oil/fat although the differences were not significant (Table 2). Feeding fat as RSB, MG or AL decreased significantly ($P<.05$) the proportion of short and medium chain fatty acids in the milk (C6:0-C14:0) (Table 3). In cows fed RSB and AL, the proportion of palmitic acid was reduced by 25% and 10.2% compared to control. However, addition of megalac increased the proportion of palmitic acid. The proportion of oleic acid (C18:1) was increased in all the treatments compared to the control irrespective of what fat source was added. Addition of fat did not change rumen VFA and blood glucose concentrations. Forage DM disappearance rates were similar from bags containing forage, forage + megalac or forage + alifat, indicating no negative effect of fat addition on forage digestion.

Conclusion

Results from this study indicate that by changing the fatty acid composition of the diet, the fatty acid profile of the milk can be changed. The changes in long chain fatty acids of milk fat by adding roasted soybeans were of the highest magnitude followed by alifat and megalac.

Table 1. Ingredient composition of experimental diets (% dry basis).

	Treatment			
Ingredient	1	2	3	4
Alfalfa silage	55.0	55.5	55.0	55.0
Dry shelled corn	28.0	24.0	23.3	23.8
Soybean meal	15.2	3.2	16.0	16.0
Roasted soybeans	-	16.0	-	-
Megalac[1]	-	-	3.9	-
Alifat[2]	-	-	-	3.4
Mono and dicalcium phosphate	1.1	1.1	1.1	1.1
Trace mineral salts	.7	.7	.7	.7

Additional Vitamin supplementation
[1]Calcium salt of palm oil
[2]Tallow product

Table 2. Effect of fat supplementation on DM intake, milk yield and milk composition.

	Treatment - Multiparous cows				Treatment - Primiparous cows			
	1 (CTL)	2 (RSB)	3 (MG)	4 (AL)	1 (CTL)	2 (RSB)	3 (MG)	4 (AL)
DM intake, kg/d	28.3	28.4	26.5	28.2	23.2[a]	23.4[a]	20.5[b]	21.9[ab]
Milk yield, kg/d	33.0	35.6	34.1	35.8	31.6	32.3	31.1	31.9
Fat, %	3.55[b]	3.53[b]	3.78[a]	3.36[b]	3.20[ab]	3.38[a]	3.42[a]	3.14[b]
Protein, %	2.97[a]	2.92[ab]	2.93[ab]	2.91[b]	3.04[a]	2.98[ab]	2.92[b]	2.92[b]
Milk fat yield, kg/d	1.18[a]	1.26[ab]	1.29[b]	1.21[ab]	.95[c]	1.10[a]	1.06[a]	1.01[b]
Milk protein yield, kg/d	.98	1.04	1.0	1.05	.90[b]	.97[a]	.91[ab]	.93[ab]

[a,b,c]Means in the same row with different superscripts differ ($P<.05$).

Table 3. Effect of fat supplementation on milk fatty acid composition (% of total fatty acids recovered) (multiparous and primiparous cows together).

Fatty acid	Treatment			
	1 (CTL)	2 (RSB)	3 (MG)	4 (AL)
C4:0	2.5[b]	2.5[b]	2.5[b]	2.6[a]
C6:0	2.1[a]	2.0[b]	1.9[c]	2.0[b]
C8:0	1.5[a]	1.4[b]	1.2[c]	1.3[b]
C10:0	3.7[a]	2.9[b]	2.6[c]	2.9[b]
C12:0	4.5[a]	3.2[b]	3.0[c]	3.3[b]
C14:0	13.6[a]	11.0[c]	10.5[d]	11.9[b]
C16:0	36.7[b]	27.4[d]	37.4[a]	32.9[c]
C18:0	10.3[c]	14.0[a]	9.8[c]	11.8[b]
C18.1	20.5[c]	27.3[a]	26.2[b]	26.5[ba]
C18:2	3.3[c]	6.1[a]	3.5[b]	2.9[d]
C18:3	1.5[c]	2.2[a]	1.5[c]	1.8[b]

[a,b,c,d]Means in the same row with different superscripts differ (P<.05).

EFFECT OF MATURITY OF ENSILED ALFALFA ON MILK PRODUCTION OF LACTATING COWS FED HIGH FORAGE DIETS

C.F. LEE and L.D. SATTER

Introduction

Earlier cutting of alfalfa will definitely lead to higher quality forage but does the improvement in quality pay for the higher cost of maintaining alfalfa fields when a more intensive cutting management is followed? The purpose of this study was to measure the milk production response in lactating cows fed alfalfa harvested under a 3-cut system (early flower) or a 4-cut system (mid bud). An agronomic study is being conducted with both cutting systems to study the impact of cutting management on alfalfa yields and longevity.

Procedures and Methods

Silage. Two alfalfa fields (first year after seeding year) were harvested on a 3-cut or 4-cut system. Cutting dates and yields for the alfalfa fields for 1989 and 1990 are shown in Table 1. Alfalfa was ensiled in bunker silos, with each cutting layered in on top of the preceding cutting. This enabled simultaneous feeding of silage from all cuts throughout the whole experiment.

Experiment 1. (Mid-lactation cows). Forty-eight mid-lactation cows were divided into two groups of 24 and fed total mixed rations containing 75% alfalfa silage (either 3-cut or 4-cut), 13.2% high moisture ear corn, 10% roasted soybeans and 1.8% of a vitamin and mineral mixture (DM basis). Individual feed consumption was not measured because cows were group fed in a free stall barn. The experimental design consisted of a two week pre-experiment period followed by an eight week treatment period. Diets were switched at the end of eight weeks,

and a short three week switchback period was run.

Experiment 2. (Early-lactation cows). The forage used was the same as in experiment 1. Nine primiparous and seven multiparous Holstein cows were fed a standard diet during the two week preliminary period. At the beginning of the third week of lactation, cows were randomly assigned to diets containing 60% alfalfa silage (either 3-cut or 4-cut), 23.2% high moisture ear corn, 15% roasted soybeans and 1.8% of a mineral and vitamin mixture (DM basis). Cows were in tie stalls and fed individually. The experimental design was a single switchback consisting of two periods lasting four weeks each.

Results

Experiment 1. Feed composition and cow response are in Tables 2 and 3. The 3-cut forage contained more fiber and less protein than the 4-cut forage. Since pretrial milk production for the two groups was nearly identical, adjustment of treatment means by covariance was not done. Cows fed 4-cut alfalfa produced 2.1 kg/day more milk than cows fed 3-cut alfalfa.

Figure 1 shows weekly milk production during the pretrial, treatment and switchback period. It is clear that cows fed the 3-cut alfalfa produced less milk. This is in contrast to the studies of Nelson and Satter where little or no difference in milk production was observed when forages similar to those in this experiment were fed to mid-lactation cows. It should be pointed out that cows in the present study were fed diets containing more forage (75% of diet DM) than in the studies by Nelson and Satter (55 to 60% of diet DM).

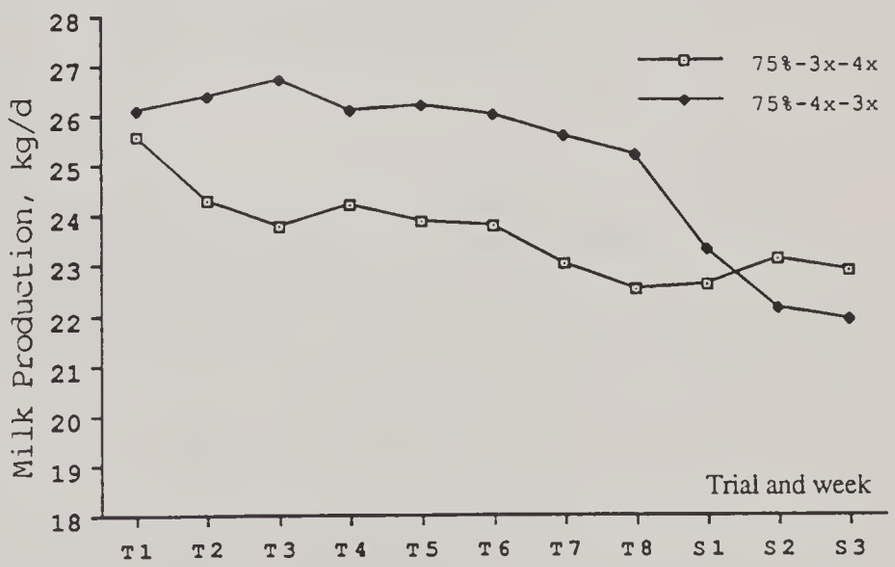


Figure 1. Milk production of mid-lactation cows fed 3-cut or 4-cut alfalfa silages in diets containing 75% (DM basis).

Experiment 2. Dry matter intake of both diets was poor for both primiparous and multiparous cows, with dry matter intake ranging between 2.5-3.0 and 2.5-3.3% of body weight for the two groups. This experiment was conducted during the summer, and feed-out rate from the silos was slow. This most likely contributed to aerobic deterioration of silage at the face of the silo. This was evidenced by lactate and acetate contents (% of DM) of 3.6 and 4.4% for 3-cut silage, and 2.7 and 3.9% for 4-cut. Dry matter intake and milk production are in Table 3.

It appears that there was a modest effect of maturity on milk production. The effect of maturity was less with the early lactation cows than with the mid-lactation cows. The mid-lactation cows were fed diets higher in forage, however. The low quality of silage in this experiment (due to aerobic deterioration) compromises the results, and care must be used in interpreting this experiment.

Conclusion

A larger reduction in milk production response was observed in this study relative to earlier studies. This may be due to the higher proportion of forage in the diet.

Table 1. Cutting dates and yields for alfalfa harvested 3x or 4x by September 1.

	1989		1990	
	Date of Harvest	Yield (ton DM/acre)	Date of Harvest	Yield (tons DM/acre)
3-cut	6/8	1.54	6/6	1.91
	7/10	1.31	7/11	1.65
	8/16	1.03	8/14	.82
	Total	3.88	Total	4.38
4-cut	5/25	1.22	5/23	1.16
	6/29	.93	6/27	1.24
Too short - Cut & left in field		0	7/26	.71
	8/22	.76	8/31	.71
	Total	2.91	Total	4.19

Table 2. Alfalfa silage composition (Expts 1 and 2) (Alfalfa harvested in 1989).

	3-cut	4-cut
Alfalfa Composition (%)		
Dry Matter	31.9	27.9
Crude Protein	17.8	19.2
Neutral Detergent Fiber	47.6	44.8
Acid Detergent Fiber	39.0	36.3
Acid Detergent Lignin	7.1	6.2

Table 3. Cow response (Expt. 1)

	3-cut	4-cut
Milk (kg/day)	23.8	25.9
4% FCM (kg/day)	23.0	24.4
Milk Fat (%)	3.70	3.54
Milk Protein (%)	3.02	3.03
Dry Matter Intake (kg/day)	20.5	20.0
Feed Efficiency (kg milk/kg DMI)	1.16	1.29
Body Weight Change (kg)	14.5	22.7
Final Body Score	3.7	4.0

Table 4. Dry matter intake and milk production (Expt. 2) (Alfalfa harvested in 1989).

	Primiparous Cows	Multiparous Cows
	----------kg/day-------	
Dry Matter Intake		
3-cut forage	12.9	15.5
4-cut forage	13.9	16.2
Milk Production		
3-cut forage	23.2	29.2
4-cut forage	24.7	29.8

UTILIZATION OF MACERATED ALFALFA FORAGE BY LACTATING DAIRY CATTLE

D.R. MERTENS, R.W. HINTZ and R.G. KOEGEL

Introduction

Harvesting alfalfa as a macerated forage mat can reduce drying time and losses associated with harvesting. Macerated alfalfa has yielded increased digestibility and available energy when forage-only diets were fed to growing sheep and goats. The effect of mat processing on the utilization of alfalfa by lactating dairy cattle fed mixed diets has not been evaluated.

Materials and Methods

Macerated and non-macerated first-crop alfalfa was harvested as silage and stored in individual polypropylene bags. The macerated silage contained 43.3% NDF and 18.4% CP, while the control silage contained 45.1% NDF and 15.7% CP. The two silages were combined with high-moisture ear corn, solvent-extracted soybean meal, minerals and salt to formulate total mixed rations containing 34% NDF and 17% CP on a dry matter basis. Rations were offered to 12 lactating Holstein cows at a level to allow an average of 10% refusal. The experiment was conducted as a two-period switchback design with 28 day experimental periods.

Results and Discussion

Dry matter intake, milk production and milk composition were not affected by maceration, but body weight gain was significantly higher for animals fed diets containing macerated alfalfa forage than for animals consuming the control forage (Table 1). Intakes were similar between treatments suggesting that differences in gain were due to tissue changes and not gut fill. Cows in this trial were in mid to late lactation and may have responded to increased energy availability by replenishing body reserves rather than increasing milk production.

Assuming constant net energy of lactation (NEL) values for concentrate ingredients (NRC, 1978), NEL's of the forages were estimated from calculated net energy output of the cows. Energy obtained from the forage component of the diet indicates that the macerated forage provided approximately 10% more NEL than did unmacerated silage (Table 2). The increase in body weight deposition and slight reduction in milk fat concentration suggest that maceration of alfalfa not only may have increased energy availability, but also may have altered rumen fermentation and increased the proportion of propionate relative to acetate production in the rumen. Research is in progress to evaluate this hypothesis.

Table 1. Performance of lactating dairy cattle fed total fed rations containing macerated or control alfalfa silages.

Silage	Milk	4%SCM[a]	GAIN[b]	DMI[c]	Fat	Protein	Lactose
	----------	-kg/d	---------		--------	-%	--------
Control	24.6	23.5	0.10	19.9	3.71	3.26	4.85
Macerated	24.2	22.4	0.58	19.6	3.56	3.20	4.74
LSD (P<05)[d]	1.5	3.9	0.26	1.0	0.27	0.15	0.23

[a]4% Solids-corrected milk production.
[b]Body weight change.
[c]Dry matter intake.
[d]Least Significant Differences presented for comparison purposes only.

Table 2. Estimates for net energy of lactation values (NEL) for macerated and non-macerated silages.

Forage		Energy Output	Total	Concentrate	Forage
Control	- DMI (kg/d)		19.92[a]	6.97	12.95
	- NEL (Mcal/d)	27.12[b]	27.12[b]	12.97[c]	14.15[d]
	- NEL (Mcal/kg)			1.86[c]	1.10[d]
Macerated	- DMI (kg/d)		19.61[a]	6.86	12.74
	- NEL (Mcal/d)	28.30[b]	28.30[b]	12.76[c]	15.53[d]
	- NEL (Mcal/kg)			1.86[c]	1.22[d]

[a]Dry matter intake.
[b]Energy output calculated from maintenance, body weight change and energy content of milk produced.
[c]NEL from concentrates calculated from NRC (1978).
[d]Forage NEL calculated by difference.

EVALUATING VARIOUS MODIFICATIONS OF THE NEUTRAL DETERGENT FIBER METHOD FOR FEEDS

D.R. MERTENS and R.W. HINTZ

Introduction

A major modification of neutral detergent fiber (NDF) analysis of feeds has been the addition of alpha-amylase treatment to remove starch which can contaminate fiber residues. The manner in which the enzyme has been used has varied widely and a number of modifications are used by various researchers and analytical laboratories. As part of an on-going project to develop a standardized NDF procedure that can be approved by the Association of Official Analytical Chemists, we evaluated several modifications to determine which of them was best suited for use as an official method for NDF.

Materials and Methods

Six modifications of the neutral detergent fiber analysis, varying only in the manner in which the alpha-amylase enzyme was added, were compared on six feeds. The procedures were:

PROCEDURE DESCRIPTION

1. 100 ml of neutral detergent solution, 0.25 ml of alpha-amylase (Novo Termamyl) added prior to the onset of boiling.
2. Samples incubated overnight in 50 ml 8M urea, 50 ml of neutral detergent solution containing twice the normal concentration of reagents added, and refluxed for 60 minutes.
3. Samples refluxed for 40 minutes in 45 ml of neutral detergent solution, an additional 55 ml of room temperature neutral detergent solution was added along with 0.1 ml of the alpha-amylase and the sample was allowed to reflux for an additional 20 minutes. After transferring fiber residues, an additional 0.1 ml of the alpha-amylase was added to the samples during the first soaking in hot water.
4. Same as procedure 3 except that the amount of alpha-amylase was doubled.
5. 100 ml of neutral detergent solution, 0.1 ml of alpha-amylase added after the onset of boiling and during the first soak after transferring residues.
6. Same as procedure 5 except that the amount of neutral detergent solution was reduced to 50 ml.

Results and Discussion

Statistically significant ($P>0.05$) differences were detected in NDF recovery among the six procedures (Table 1). Preincubation in 8M urea provided the lowest NDF residue recoveries, even on samples that contain little or no starch. Reports in the literature suggest that 8M urea may solubilize hemicellulose which may explain the consistently low fiber recoveries observed with that technique. The highest NDF residue recoveries were obtained with procedure 1 and suggest that either the quantity of enzyme used or the timing of its application may not provide complete removal of starch contamination. Although statistically significant, differences among the remaining procedures were of small magnitude. The smallest standard deviation among analysts was obtained by procedure 6. This procedure provided NDF recoveries that do not appear to be contaminated by starch, produced the smallest standard deviation among analysts and reduced reagent costs by 50%. For these reasons, we decided to use procedure 6 in developing a standard technique.

Table 1. Neutral detergent fiber concentrations of six feeds as determined by six analytical procedures averaged over three analysts and two grind sizes.

	PROCEDURE[a]					
	1	2	3	4	5	6
Sample	% NDF	% NDF	% NDF	% NDF	% NDF	% NDF
Corn Silage	43.23	41.25	42.86	42.38	42.84	42.52
Barley Grain	20.79	15.81	17.95	17.47	18.80	18.04
Grass Hay	61.69	60.57	61.91	1.82	61.97	61.40
Soybean Meal	12.43	11.78	12.64	12.16	12.51	12.48
Alfalfa Hay	32.90	31.81	31.87	31.43	32.07	31.91
Corn Grain	9.82	8.61	8.91	8.52	9.10	8.60
Average	30.14	28.30	29.36	28.96	29.55	29.16
Std. Dev.[b]	0.45	0.39	0.55	0.44	0.49	0.31
LSD (P<0.05)[c]	0.26					

[a]Procedures:
1 = 0.25 ml Termamyl prior to boiling.
2 = Overnight incubation in 8M urea.
3 = 0.1 ml Termamyl added 40 minutes after boiling and at filtering.
4 = Same as procedure 3 except Termamyl dosage was doubled.
5 = 0.1 ml Termamyl added 1 minute after boiling and at filtering.
6 = Same as procedure 5 except that only 50 ml of ND solution was used.
[b]Standard deviation among 3 analysts averaged over feeds.
[c]Least significant difference (P<0.05) for comparing NDF means between two procedures.

MODELING DIFFERENCES IN DIGESTION AND PASSAGE AMONG LARGE AND SMALL RUMINANTS

D.R. MERTENS

Introduction

To survive, propagate and be productive, animals must meet their energy needs for maintenance, activity, reproduction and lactation. Thus, energy demand becomes the driving force for feed intake. The major constraints on digestible energy intake are: the potential extent and rate of digestion (kd), rate of passage (kp) and gut capacity (CAP). Understanding the processes by which the diet and the animal interact to meet energy requirements is enhanced by integration of quantitative information about the animal's capacity and the kinetics of digestion and passage. The objectives of this research were to develop a model of digestion and passage which can predict digestibility and ruminal capacity of NDF and use the model to determine relationships between the energy demands of large and small ruminants and kd, kp and CAP.

Materials and Methods

The model developed was a refinement of the model of Mertens and Ely (1979) and is based on the use of NDF to fractionate feeds into kinetically distinct components. To the original model was added microbial synthesis and digestion of microbial cells, pools of digestible and indigestible neutral detergent soluble matter, and direct correlation of the various kinetic processes of passage (rates of particle size reduction due to rumination, rates of escape from the rumen and rate of passage through the intestines). Data obtained from published literature were used to define realistic

ranges for the parameters of the model. Sensitivity analysis was used to compare the effects of kd, kp and CAP on the digestibility, intake and capacities for a 60 kg sheep and 600 kg cow at one and two times maintenance requirements. Digestible energy requirements were calculated as .155 Mcal times body weight raised to the .75 power (NRC, 1989).

Results and Discussion

The model predicts that, if large and small ruminants have similar CAP and kp, smaller animals will have to select less fibrous or more rapidly digesting feeds to meet their energy demands (Table 1). It appears that a small animal at 1X maintenance would have to select a diet that could meet the energy requirements of a large ruminant at 2X maintenance. These model results agree with observations in domestic and wild ruminants that small animals are more selective and prefer feed sources that are lower in fiber than those selected by large ruminants.

Conversely, the model predicts that if large and small ruminants have similar CAP and are constrained to diets of similar kd, smaller animals must have a more rapid kp than large ruminants to consume and process enough feed to meet their energy needs (Table 2). There is limited data to confirm this prediction under conditions that are comparable to the situations that were modeled, but published literature suggests that sheep typically have faster kp than cattle. The faster kp of small ruminants results in lower digestibilities for fibrous diets than when the same diets are consumed by large ruminants. This agrees with the general observation that sheep have lower digestibilities of fibrous diets compared to cattle. Results in Table 2 indicate that animals would have to increase rate of passage when fed slower-digesting diets to meet their energy demands. This somewhat counterintuitive prediction suggests that throughput is more critical than efficiency of digestion in meeting the animal's requirements.

Conclusions

The model provides quantitative information about the interactions between characteristics of the animal and the diet that affect the utilization of feeds and the performance potential of animals. It is useful as a theoretical framework for explaining the differences in digestibility, intake and passage among animal species and may provide the means for more accurately relating the feed evaluation data obtained from sheep trials to their use in assessing nutritive values for cattle. Due to lower expense and amount of feed required, sheep are used most often to evaluate feeds. Use of this or similar models similar to improve the accuracy for using sheep data to estimate feed value for dairy cattle would have significant practical utility.

Table 1. Rate of digestion required to meet the digestible energy requirements of animals with various ruminal capacities and rates of passage (kp).

	Cattle Rumen Capacity[a]			Sheep Rumen Capacity[a]		
kp	1.1	1.3	1.5	1.1	1.3	1.5
			1X maintenance requirements			
0.03	.068	.062	.057	.087	.082	.077
0.05	.061	.056	.051	.077	.073	.069
0.07	.058	.053	.049	.073	.069	.066
			2X maintenance requirements			
0.03	.092	.086	.081	.115	.109	.104
0.05	.080	.076	.072	.093	.089	.086
0.07	.075	.072	.068	.086	.083	.081

[a]Percent of body weight.

Table 2. Rate of passage required to meet the digestible energy requirements of animals with various ruminal capacities fed forages with various rates of digestion (kd).

	Cattle Rumen Capacity[a]			Sheep Rumen Capacity		
kd	1.1	1.3	1.5	1.1	1.3	1.5
			2X maintenance requirements			
0.09	.032	.025	.019	.055	.048	.042
0.10	.024	.018	.012	.041	.036	.032
0.11	.020	.015	.010	.037	.032	.028

[a]Percent of body weight

IN VITRO DETERMINATION OF RUMINAL PROTEIN DEGRADABILITY USING N-15 AMMONIA TO CORRECT FOR MICROBIAL N-UPTAKE

A. HRISTOV and G. A. BRODERICK

Introduction

Ruminal protein degradation determines in part the value of feed proteins to lactating dairy cows and other ruminants. Recent interest in resistant feed proteins, particularly heat-treated proteins such as roasted soybeans, has stimulated the need for rapid in vitro methods to quantify ruminal protein degradation. Common in vitro methods for estimating protein degradability, based on NH_3 release, have proven inaccurate because ruminal microbes take up as well as release NH_3. As a result, degradabilities are underestimated and unreliable. Previously, we approached this problem by using an inhibitor in vitro (IIV) system which contained chemicals that prevented microbial N-uptake and allowed quantitative recovery of protein degradation products (Broderick, Brit. J. Nutr. 58:463, 1987). The purpose of this study was: 1) to develop a reliable in vitro method for quantifying feed protein degradability using ^{15}N-labeled NH_3 to correct for microbial uptake of protein degradation products; and 2) to determine if inhibitors in the IIV system significantly altered observed degradation rates.

Materials and Methods

Whole rumen contents were taken from a cannulated donor cow 1.5-2.0 hours after the morning feeding and squeezed through 2 then 8 layers of cheesecloth. To obtain some attached microbes, the solids remaining after squeezing were washed four times with a total volume of warm (39°C) buffer equal to the volume of strained rumen fluid (SRF) obtained originally. The mixture of SRF and buffer-wash was poured into a graduated cylinder and held at 39°C. After 30 min., the top layer containing gas-suspended feed particles was removed by vacuum aspiration, and the remaining, microbe-rich liquor was mixed and used as the inoculum. Inoculum, 240 ml, was dispensed into stirrer flasks predosed with 120 ml buffer and reducing solution (final dilution: 2 parts buffer-1 part rumen fluid) containing $(^{15}NH_4)_2SO_4$, 990mg/flask of pectin, and 737 mg/flask of a carbohydrate mixture (66% soluble starch, 16% maltose, 9% glucose and 9% sucrose). Incubations were conducted for 6 hours. After 3 hours, another 737 mg of the carbohydrate mixture and 2 ml hydroxide solution (1.5

N KOH and 1.5 N NaOH-2:1) were added to each flask. All manipulations were carried out under CO_2 free of O_2. The microbial activity at the end of the incubation was stopped by chilling on ice and adding formalin (1% of the volume). Samples were analyzed for Kjeldahl-N, NH_3-N and total amino acids, and for ^{15}N-enrichment of NH_3-N, microbial-N and total solids-N. Proteins tested were casein, solvent soybean meal (SSBM), expeller soybean meal (ESBM), low-solubles fish meal (LSFM), high-solubles fish meal (HSFM), corn gluten meal (CGM), and roasted whole soybeans (RSB); all proteins previously were analyzed for degradation in the IIV system. All proteins and the control (with no protein added) were incubated in triplicate in three studies and in duplicate in one study (n = 11). Degradability was computed based on dilution of ^{15}N in the NH_3-N pool, after correction for NH_3-N and non-NH_3-N incorporation in the microbes (relative to the control).

Results and Discussion

Figures 1 and 2 describe the metabolism of NH_3-N and microbial growth during the 6 hour in vitro incubations. Greater microbial degradation of casein and SSBM resulted in greater accumulation of NH_3-N compared to the more resistant proteins (free NH_3 released and remaining, Figure 1). With casein, less NH_3-N was incorporated by the microbes—70.6% of control, compared to 92.3-109.2% of control for the other proteins. Figure 2 shows that greater protein degradability resulted in greater incorporation into microbial protein of N from other than NH_3; this was probably N directly incorporated from peptides and amino acids. Microbial N concentration for all proteins was greater than the control (Figure 2). Degradabilities are shown on Table 1. A simple correction for NH_3 recovery using ^{15}N-NH_3 did not prove satisfactory as a

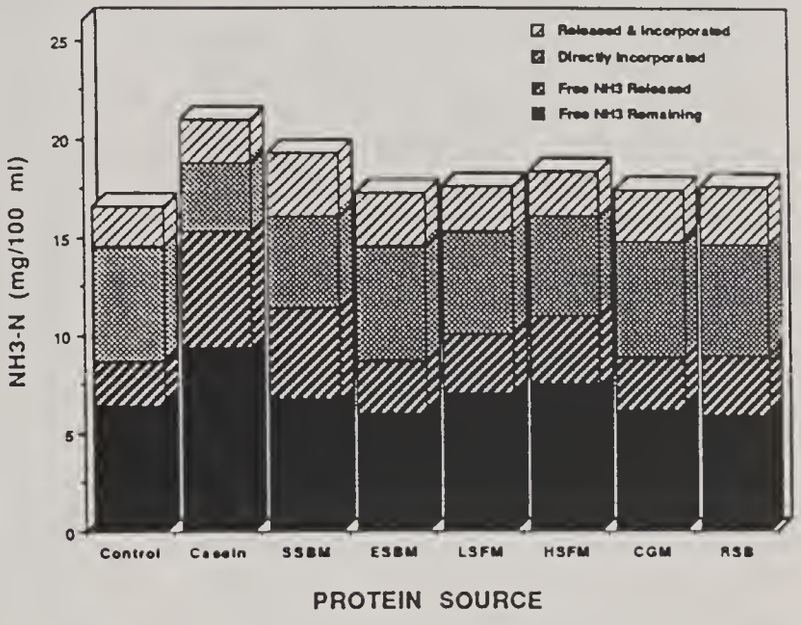


Figure 1. Metabolism of NH_3-N during 6-hour in vitro incubations.

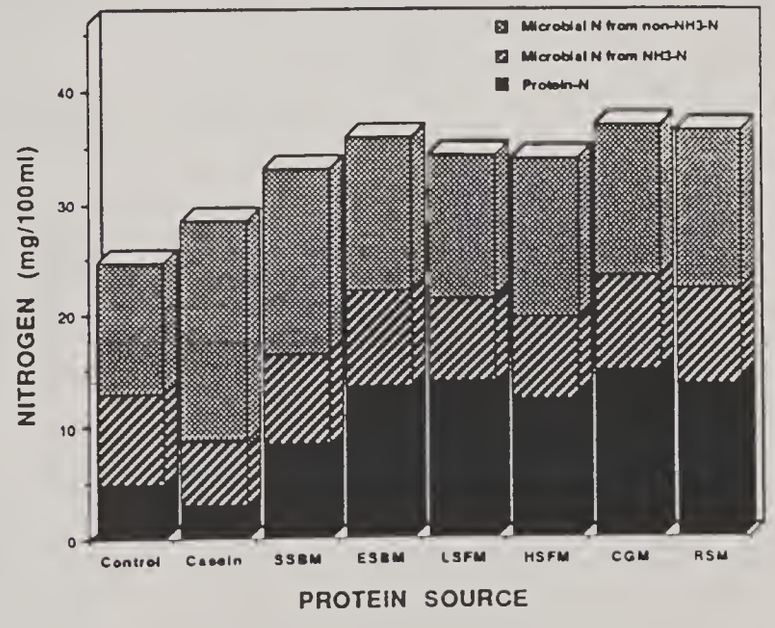


Figure 2. Origin of N in "total solids" after 6-hour incubations.

method for computing protein degradation. Casein was almost completely degraded in 6 hours (mean = 94%); LSFM was least degradable (mean = 15%). Fractional degradation rates, computed from 6-hour extents of degradation, were of similar magnitude but generally greater than those obtained by the IIV method. Rates obtained by the ^{15}N method for casein, SSBM, ESBM, CGM and RSB averaged 60% greater than IIV rates. However, rates for LSFM and HSFM were lower. Oil associated with the fish meals may have reduced degradation by inhibiting microbial growth in the ^{15}N incubations; this inhibition would not influence IIV results. Estimated ruminal escapes were similar to values computed using IIV rates. Although extents of ruminal escape estimated for SSBM, both fish meals and CGM were lower than values given by the 1988 Dairy NRC Bulletin, the rate obtained by the ^{15}N method for casein (.457/h) was almost identical to the rate (.462/h) observed in vivo in an earlier study.

Summary and Conclusion

An in vitro method, based on use of ^{15}N-NH_3 to correct for microbial uptake of protein degradation products, was developed to serve as a more theoretically sound, standard procedure with which to compare other in vitro tests for protein degradability. The method gave reliable estimates of rate and extent of in vitro degradation, and in vivo ruminal protein escape.

Table 1. Comparison of in vitro ruminal degradation rates and escapes estimated using the N-15 and the IIV methods.

	Protein source						
	Casin	SSBM	ESBM	LSFM	HSFM	CGM	RSB
N-15 Method							
In vitro degradation (6 h), %	93.6	65.6	24.0	15.4	29.5	20.3	32.1
(SE, n=11)	(2.86)	(2.16)	(1.21)	1.65)	(2.32)	(1.73)	(1.86)
Rate of degradation, /h	.457	.178	.046	.028	.058	.038	.065
Estimated escape, %	12	25	57	68	51	61	48
IIV Method							
Rate of degradation, /h	.307	.137	.030	.034	.066	.017	.045
Intercept (B), %	99.2	98.0	97.7	97.8	97.2	95.4	96.5
Estimated escape, %	17	30	65	63	46	75	56

RUMINAL PROTEIN DEGRADATION RATES ESTIMATED BY NONLINEAR REGRESSION ANALYSIS OF MICHAELIS-MENTEN IN VITRO DATA

G.A. BRODERICK and M.K. CLAYTON

Introduction

Determination of the rate and extent of ruminal degradation is critical when evaluating dietary proteins for feeding productive ruminants such as lactating dairy cows. Rates of protein degradation have been estimated by in situ techniques, and by in vitro methods using rumen inoculum or commercial enzymes. Previously, we have estimated fractional rates of microbial protein degradation, k_d, using short-term incubations in an inhibitor in vitro (IIV) system and computed extents of protein escape based on typical rates of ruminal passage. The IIV approach correlated well with in situ and in vivo results with protein concentrates, particularly soybean meals and whole roasted soybeans. However, the IIV procedure has several significant limitations, notably imprecision in determining rates for slowly degraded proteins and for proteins with high "background" levels of ammonia and amino acids (especially alfalfa forages). The objective of the present research was to develop and test a Michaelis-Menten method, in which fractional degradation rate, k_d, would be estimated as the tangent through the origin of the velocity vs. [S] curve, and determined from the ratio of maximum velocity to the Michaelis constant (i.e., $k_d = V_{max}/K_m$). This approach overcomes several limitations of the limited substrate procedure and may yield more reliable estimates of degradation rates.

Materials and Methods

Michaelis-Menten incubations were conducted with different protein sources using our basic in vitro method (Broderick, Brit. J. Nutr. 58:463-475. 1987). Six to eight protein concentrations were weighed into tubes in amounts equivalent to: 1.5, 3.0, 4.5, 6.0, 9.0, 12, 18 and 36 mg N/tube. Protein sources were then wetted at 39°C with 5-ml McDougall's buffer/tube for 1-h prior to incubation. Twelve to 16 blank tubes were used in each incubation. Incubations begun by adding 10-ml inoculum/tube (total 15 ml/tube with 4.5 ml rumen fluid equivalent). Except for a study comparing 1-h and 2-h, incubations were conducted for 2-h at 39°C. Amounts of protein degraded and remaining undegraded were computed from amounts of N released as ammonia and total amino acids. Ammonia and free amino acid contents of protein sources prior to incubation were negligible, except for alfalfa hays which had 8.06-8.96% of total N as ammonia and amino acids; degradations for alfalfa hays were corrected for 0-h degraded N. The Marquardt nonlinear regression procedure of SAS was used to fit the Integrated Michaelis-Menten Model (IMM), comprised of the equation:

$$S_t = S_o - t \times k_d \times K_m + K_m \times \ln (S_o/S_t),$$

where S_o = the amount of protein added at 0-h, S_t = the amount of protein remaining undegraded after incubation, and $k_d = V_{max}/K_m$.

Results and Discussion

Generally, more rapid degradation rates were obtained with the IMM method than with the IIV limited substrate technique. Fractional rates obtained by the IMM (2-h) and IIV methods were, respectively, .989 and .395/h, .134 and .140/h, and .037 and .024/h, for casein, solvent soybean meal (SSBM), and expeller soybean meal (ESBM). Rates of degradation obtained using 2-h incubations had lower standard errors than those obtained using 1-h incubations; 2-h rates were not significantly different from 1-h rates, suggesting end-product inhibition was not significant

at 2-h. The IMM and IIV methods were used to determine degradation rates for 12 protein sources: casein, bovine serum albumin (BSA), 2 samples of alfalfa hay, and 4 samples each of SSBM and ESBM. Statistical analysis of IMM rates revealed significant differences among the 12 protein sources. Casein was degraded most rapidly (.827/h), and the 4 ESBM samples most slowly (.050 to .098/h). Degradation rate for serum albumin was .135/h; rates for SSBM and alfalfa hays ranged from .160 to .208/h. Degradation rates estimated using the Michaelis-Menten method were more rapid than those obtained with the IIV limited substrate approach.

Degradation rates obtained by the two methods were used with a typical DM ruminal passage rate (k_p = .06/h) to compute ruminal protein escapes (Table 1). More rapid degradation rates obtained by the IMM approach yielded lower escapes than those obtained with the IIV technique. IMM-based estimates may be more reliable. Although the 13% escape obtained for casein from the IIV rate approximates in vivo reports of about 10%, the passage rate of .06/h used in its computation may be too slow. Casein probably flows with the liquids which pass out of the rumen at about two times faster than DM. A ruminal passage rate of k_p = .12/h yielded escapes of 13 and 23% at the degradation rates of .83 and .40/h obtained by IMM and IIV methodology. IMM-based estimates for alfalfa hay of 22-25% (Table 1) were more consistent with the UIP value of 28% ruminal escape assigned to alfalfa hay in the 1988 Dairy NRC. Mean ruminal escapes of 27 and 24% (Table 1) predicted for SSBM protein from IIV and IMM data, are lower than the 35% escape reported by the 1988 NRC; the NRC has not reported ruminal protein escapes for BSA or ESBM.

Summary and Conclusion

The Michaelis-Menten approach, where fractional rates of protein degradation are estimated using nonlinear regression determination of the ratio: $k_d = V_{max}/K_m$, offers a reliable alternative for determining ruminal degradability of feed proteins. This approach yielded statistically sound and reproducible estimates of degradation rate, and biologically meaningful estimates of ruminal protein escape, for casein, alfalfa hay, SSBM and ESBM.

Table 1. Estimated ruminal protein escapes (%) computed for 12 protein sources from degradation rates determined by the limited substrate (IIV) and Integrated Michaelis-Menten Models (IMM).

Protein source	N content (% DMB)	Limited Substrate[1] Intercept antilog (B)	Limited Substrate[1] Est. escape[3]	Integrated Michaelis-Menten[2] Intercept antilog (B)	Integrated Michaelis-Menten[2] Est. escape[3]
		(%, total N)			
Casein	15.6	101.9	13	100	7
BSA	15.9	106.9	31	100	30
SSBM (1/84)	7.5	99.6	28	100	22
SSBM (6/84)	7.4	102.0	27	100	27
SSBM (7/84)	7.4	101.4	27	100	23
SSBM (11/84)	8.2	102.9	27	100	25
ESBM (1/84)	7.5	97.8	55	100	45
ESBM (6/84)[4]	7.2	97.5	56	100	49
ESBM (7/84)	7.2	97.4	53	100	42
ESBM (11/84)[4]	7.2	96.4	57	100	38
Alfalfa Hay (10/83)	3.6	74.3	37	91.9	22
Alfalfa Hay (1/84)	3.1	73.8	40	91.0	25

DMB = dry matter basis; BSA = bovine serum albumin; SSBM = solvent soybean meal; ESBM = expeller soybean meal.

[1]Intercept antilogs (B) and estimated rumen escapes from limited substrate model were reported earlier (Broderick, Brit. J. Nutr. 58:463-475, 1987).

[2]Intercept antilogs (B) for the Integrated Michaelis-Menton Model were assumed = 100% because 0-h ammonia plus TAA accounted for < 1% of total N except for alfalfa. Ammonia plus TAA at 0-h accounted for 8.06 and 8.96% of total N for the alfalfa hays.

[3]Estimated Escape, % = B X [k_p/(k_p + k_d)], where k_d = degradation rate determined by either the IIV or IMM methods, and k_p is assumed = .06/h.

[4]Results from only three of four incubations were used because of non-convergence.

U.S. DAIRY FORAGE RESEARCH CENTER ANNUAL DAIRY OPERATIONS REPORT, FEBRUARY 1991

L.L. STROZINSKI

The research center herd count has remained relatively stable over the past year and currently stands at 535 (263 cows and 272 herd replacements). We are currently milking 225 cows which are yielding an average of 65 pounds of milk per day. I am pleased to report that our DHIA rolling herd average has risen by 2000 pounds of milk during the past year. Our present average is 18,450 pounds of milk, 660 pounds of fat and 570 pounds of protein. The reproductive performance of the herd has slipped in the past year but remains acceptable with our average days open at 104 and a calving interval at 12.7 months. Our average age at first calving remains at 24 months and I am extremely pleased with the quality and size of our first calf heifers. First calf heifers now make up 30% of our milking herd. The average age of our milking herd has increased slightly from 48 to 51 months which has been one of our goals.

Research usage of the herd continues to be high through the year, as it should be. Presently, 65% of the milking herd are on research trials along with 21 heifers. There is a constant demand for fresh cows to be placed on studies through the year and seldom does a fresh cow go unused for research. Many cows are assigned to more than one research trial during a given lactation and some projects are superimposed on others.

We have continued to work cooperatively with various university departments throughout the year. We have provided numerous bull calves for studies on campus. We continue to work with the State Laboratory of Hygiene on a lead project and are now working with the School of Veterinary Medicine on a milk fever project.

I am happy to report that after over a year of operating the dairy unit with vacant positions, the unit is now fully staffed. Having enough employees to adequately cover the work load has been a welcome change in our operation and I think is reflected in the service we provide and the performance of the herd.

This fall the farm hosted a soybean roasting seminar and demonstration. This event was attended by 225 feed processors and producers from Wisconsin and neighboring states.

U.S. DAIRY FORAGE RESEARCH CENTER
ANNUAL FIELD OPERATIONS REPORT, FEBRUARY 1991

R.P. WALGENBACH

Several personnel changes occurred at the research farm in 1990. Brad Venuto left the Farm Managers position and joined Dr. Dick Smith's program to pursue a Ph.D. in Plant Breeding. Brad has done an outstanding job in his managing of the research farm and assisting in a diverse assortment of research projects. A major project that Brad initiated was a Field Record Keeping and Management program. This program will be an invaluable tool for record keeping, not only at the Prairie du Sac location but most likely at many other research stations within Wisconsin and other states as well. In Brad's absence, Dave Spracher and Harry Enders did an outstanding job of keeping the farm production and research projects running smoothly. Melvin Luetscher, a tireless worker, valued employee, and friend of all retired this year. Melvin's disposition and willingness to help whenever asked helped to establish a very positive atmosphere of cooperation at the farm. We will miss you, Melvin! With Melvin's retirement, a new Federal Position (Maintenance Mechanic) was established at the Prairie du Sac location. Our farm facility is beginning to age and repair and maintenance of farm and field equipment and facilities is now a high priority. Bob Hager has been hired as the Maintenance Mechanic and we welcome him. Stuart Pergande joined the field crew this past year and we welcome Stu. Finally, Rick Walgenbach moved into the farm managers position. This position will have overall responsibility for animal, field, and maintenance operations at the farm and have a research component of about one third FTE.

The 1990 cropping season began with somewhat below normal spring temperatures which delayed some field activities. First harvest alfalfa yields were affected by this cool weather and apparent maturity was delayed. The summer season saw a return to much needed rain which contributed to excellent forage yields and excess forage production. Corn yields were fair in 1990. The cool, late spring delayed development while moisture was adequate, heat unit accumulation was lower than it has been in past years. Compaction and continuous corn on some of our fields are becoming a concern. Plans are being developed to look at long term cropping practices and rotations to improve productivity. A new forage handling system utilizing a transfer wagon and trucks to haul forage from field to storage facilities is being purchased for the farm. Construction of a new chemical storage and handling facility should begin sometime this summer and major repair of barn roofs has been approved for funding.

PUBLICATIONS

AMJED, M. 1990. Attempts to improve sugarcane bagasses and pith as energy sources for ruminant animals. Ph.D. Thesis, Univ. of MN, p. 74 (with H.G. JUNG).

AMJED, M., J.D. DONKER, H.G. JUNG and R.A. BLANCHETTE. 1990. Effect of incubating sugarcane bagasse, pith from bagasse and wheat straw with the lignolytic fungus Phanerochaete chrysosporium. J. Anim. Sci. 68 (Suppl. 1):581 (abstract).

BIDLACK, J.E. 1990. Cell-wall components and lignin biosynthesis in forages. Ph.D. Thesis. Iowa State University, Ames. (D.R. BUXTON and R.M. SHIBLES, co-major professors).

BRODERICK, G.A. and L.D. SATTER. 1990. High bypass protein may be concern in feeding alfalfa forage-based diets. Feedstuffs, September 10, 1990.

BRODERICK, G.A. and L.D. SATTER. 1990. Importance of by-pass protein for lactating cows fed diets based on alfalfa silage. pp. 149-160. IN: "Symposium International sur L'Enzilage D-Herbe", Rouyn-Noranda, Quebec.

BRODERICK, G.A. and M.K. CLAYTON. 1990. Ruminal protein degradation rates estimated by nonlinear regression analysis of Michaelis-Menten in vitro data. J. Dairy Sci. 73(Suppl. 1):216 (abstract).

BRODERICK, G.A., D.B. RICKER and L.S. DRIVER. 1990. Expeller soybean meal and corn by-products versus solvent soybean meal for lactating dairy cows. J. Dairy Sci., 73, 453-462.

BUCKMASTER, D.R., C.A. ROTZ and J.R. BLACK. 1990. Value of alfalfa losses on dairy farms. Trans. ASAE 33(2):351-360.

BUCKMASTER, D.R., D.R. MERTENS, J.A. BLACK and C.A. ROTZ. 1989. Feed allocation and animal utilization, Chapter 6, p. 90-116. IN: C.A. Rotz (ed.) DAFOSYM Reference Manual, Agric. Engr. Dept., Michigan State Univ.

BUXTON, D.R. 1990. Alfalfa hay quality—It's feeding value. p. 31-43. IN: Proc. Nevada 1990 Alfalfa Symposium. Sparks, NV 7-8 Feb. University of Nevada, Reno.

BUXTON, D.R. 1990. Cell-wall components in divergent germplasm of four perennial forage grass species. Crop Sci. 30:402-408.

CADORNIGA-VALINO, C. 1990. Protein supplementation of high alfalfa silage diets for dairy cows. MS Thesis, University of Wisconsin, Madison (with L.D. SATTER).

CASLER, M.D. and R.R. SMITH. 1990. Why should plant germplasm explorers collect passport data? Agron. Abst. p. 84.

CHEN, G. and J.B. RUSSELL. 1990. Transport and deamination of amino acids by a gram-positive, monensin-sensitive ruminal bacterium. Appl. Environ. Microbiol. 56:2186-2192.

CHERNEY, D.J.R., D.R. MERTENS and J.E. MOORE. 1990. Intake and digestibility by wethers as influenced by forage morphology at three levels of forage offering. J. Anim. Sci. 68:4387-4399.

CHERNEY, D.J.R., D.R. MERTENS and D.R. MOORE. 1990. Morphology, fiber composition and mean particle diameter relationships in ground barley and oat forages at different ages. Anim. Feed Sci. Tech. 31:65-78.

CHOW, J.M. and J.B. RUSSELL. 1990. Effect of ionophores and pH on growth of Streptococcus bovis in batch and continuous culture. Appl. Environ. Microbiol. 56:1588-1593.

CHOW, J.M. and J.B. RUSSELL. 1990. Effect of pH on monensin and lasalocid binding to Streptococcus bovis JB1. Ann. Meeting Amer. Soc. Microbiol., Anaheim, CA, May 13-17, 1990 (abstract).

DADO, R.G., D.R. MERTENS and G.E. SHOOK. 1990. Theoretical metabolizable energy and protein requirements for milk component production. J. Dairy Sci. 73(Suppl. 1):248.

DADO, R.G., D.R. MERTENS, G.A. BRODERICK and R.W. HINTZ. 1990. Effect of protein source on optimal dietary neutral detergent fiber levels for cows in early lactation. J. Dairy Sci. 73(Suppl. 1):126.

DADO, R.G., D.R. MERTENS, G.A. BRODERICK and R.W. HINTZ. 1990. Effects of supplemental fat and resistant protein in alfalfa silage diets fed to early lactation cattle. J. Dairy Sci. 73(Suppl. 1):126 (abstract).

DADO, R.G., G.E. SHOOK and D.R. MERTENS. 1990. A merit function for yield traits based on income over feed cost. J. Dairy Sci. 73(Suppl. 1):253.

DHIMAN, T.R. and L.D. SATTER. 1990. Response to intra-ruminal infusion of propionic acid and abomasal infusion of protein in lactating cows fed alfalfa silage. J. Dairy Sci. 73 (Suppl. 1):124 (abstract).

DHIMAN, T.R. and L.D. SATTER. 1990. Response to postruminal infusion of glucose or protein during early lactation in dairy cows fed alfalfa silage. J. Dairy Sci. 73 (Suppl. 1):124 (abstract).

DHIMAN, T.R., C.M. WACEK and L.D. SATTER. 1990. Effect of high inoculation rates on alfalfa silage fermentation and animal performance. J. Dairy Sci. 73 (Suppl. 1):194 (abstract).

EDE, R.M., J. RALPH and L. MAIN. 1990. *Syn* and *anti* lignin model quinone methides. Aspects of their synthesis, characterization, and differential reactivity with amines. J. Wood Chem. Technol. 10(1), 101-110.

ENDRES, C.S. 1990. Stability and Selection Response for Digestibility of Stems in Divergent Alfalfa Populations. Ph.D. Thesis. University of Wisconsin, Madison, WI, p. 128 (with R.R. SMITH).

ENDRES, C.S. and R.R. SMITH. 1990. Stability of quality traits in stems of divergent alfalfa populations. Agron. Abst. p. 87-88.

FOX, D.G., C.J. SNIFFEN, J.D. O'CONNOR, J.B. RUSSELL and P.J. VAN SOEST. 1990. The Cornell net carbohydrate/protein system for evaluating cattle diets. Cornell Univ. Agric. Experiment Station Bulletin #43, Ithaca, NY.

FOX, D.G., J.D. O'CONNOR, C.J. SNIFFEN, P.J. VAN SOEST, J.B. RUSSELL, W. CHALUPA and K. HOUSENECHT. 1990. Using the Cornell Net Carbohydrate-Protein System to predict the effects of metabolic modifiers on the metabolizable energy and protein requirements of growing cattle, p. 28-33. Proc. Cornell Nutrition Conference for Feed Manufacturers, Rochester, NY.

GRANT, R.J. and D.R. MERTENS. 1990. Effect of in vitro fermentation technique upon kinetics of neutral detergent fiber digestion. J. Dairy Sci. 73(Suppl. 1):129.

GRANT, R.J. and D.R. MERTENS. 1990. Impact of forage type, corn starch addition and buffer pH upon in vitro digestion kinetics of neutral detergent fiber. J. Anim. Sci. 68(Suppl. 1):588.

HABY, V.A., M.P. RUSSELLE and E.O. SHOGLEY. 1990. Testing soils for potassium, calcium, and magnesium. p. 181-227. IN: R.L. Westerman (ed.) Soil testing and plant analysis. 3rd ed. Soil Sci. Soc. Am., Madison, WI.

HALIM, R.A., D.R. BUXTON, M.J. HATTENDORF and R.E. CARLSON. 1990. Crop water stress index and forage quality relationships in alfalfa. Agron. J. 82:906-909.

HATFIELD, R.D. 1990. Physiological changes and metabolic events that reduce lignocellulose utilization. p. 91-98. (D.E. Akin, L.G. Ljungdahl, J.R. Wilson, P.J. Harris, ed.). Microbial and plant opportunities to improve lignocellulose utilization by ruminants. Elsevier, New York.

HATFIELD, R.D. and J. RALPH. 1990. Pyrolysis-GC-MS of Plant Cell Wall Materials. Plant Physio. 92(5):440.

HELM, R.F., J. RALPH and R.D. HATFIELD. 1990. Dibutyltin Oxide-Mediated Acylation of D-Xylopyranosides. 200th ACS National Meeting, August 26-31, 1990, Washington, DC. Paper CARB-24.

HOFFMAN, P.C., R.R. GRUMMER, R.D. SHAVER, T.R. DRENDEL, and G.A. BRODERICK. 1990. Effects of supplemental fat and resistant protein in alfalfa silage diets fed to early lactation cattle. J. Dairy Sci. 73(Suppl. 1):243 (abstract).

HUNCZAK, J.J., J.R. GEORGE and D.R. BUXTON. 1990. In vitro dry matter disappearance responses to early defoliation treatments of switchgrass. p. 145. IN: Agronomy Abstracts. ASA, Madison, WI.

JINA, A.N., J. RALPH and C.E. BALLOU. 1990. Synthesis of affinity ligands and radioactive probes for isolation and study of myo-Inositol 1,4,5-trisphosphate binding proteins. Biochemistry 29(21), 5203-9.

JUNG, H.G. 1990. Effects of lignin linkage and hydrogen bonding on cellulose degradation in model systems. J. Anim. Sci. 68(Suppl. 1):588 (abstract).

JUNG, H.G. and J. RALPH. 1990. Phenolic-carbohydrate complexes in plant cell walls and their effect on lignocellulose utilization. Trilateral Workshop on "Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants."

JUNG, H.G. and J. RALPH. 1990. Phenolic-carbohydrate complexes in plant cell walls and their effect on lignocellulose utilization. Pages 173-182. IN: D.E. Akin, L.G. Ljungdahl, J.R. Wilson and P.J. Harris (eds.). Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants. Elsevier Scientific Publ. Co., Inc., New York, NY.

JUNG, H.G. and M.D. CASLER. 1990. Lignin concentration and composition of divergent smooth bromegrass genotypes. Crop Sci. 30:980-985.

JUNG, H.G. and S.C. SHALITA-JONES. 1990. Variation in the extractability of esterified p-coumaric and ferulic acids from forage cell walls. J. Agric. Food Chem. 38:397-402.

JUNG, H.G., P. AMAN and H. GRAHAM. 1990. Profile of fibre composition in lucerne hay and rumen digesta as influenced by particle size and time after feeding. J. Sci. Food Agric. 51:411-416.

KEPHART, K. D., D.R. BUXTON and R.R. HILL, JR. 1990. Digestibility and cell-wall components of alfalfa selected for divergent herbage lignin concentration. Crop Sci. 30:207-212.

KEPHART, K.D., L.G. HIGLEY, D.R. BUXTON and L.P. PEDIGO. 1990. Cicer milkvetch forage yield, quality, and acceptability to insects. Agron. J. 82:477-483.

KOEGEL, R.G., F.J. FRONCZAK, K.J. SHINNERS, R.J. STRAUB and G.J. CLOSE. 1990. Pressure measurement in a continuous press. Trans. ASAE. 33(4):1071-1074.

KOEGEL, R.G., F.J. FRONCZAK, S.T. WALL, K.J. SHINNERS and R.J. STRAUB. 1990. Radial blade and anvil forage cutterhead. Trans. ASAE. 33(4):695-698.

KOEGEL, R.G., R.J. STRAUB, K.J. SHINNERS, G.A. BRODERICK and D.R. MERTENS. 1990. Mechanical and thermal treatments of forage crops for improving properties. Proc. Ag-Eng 1990, Berlin, Germany. p. 150-151.

KOEGEL, R.G., R.J. STRAUB, W.P. DELZER and J.Y. KIM. 1990. Design of a reciprocating press for solid-liquid separation of dairy cattle waste. Proc. 6th Intl. Symp. on Agr. and Food Proc. Waste, Chicago. p. 269-2974.

KRAUS, T.J. 1990. Development of a crushing-impact forage maceration device. M.S. Thesis. Univ. of Wisconsin-Madison (with R.G. KOEGEL).

KRAUS, T.J., R.G. KOEGEL and K.J. SHINNERS. 1990. Impact maceration of alfalfa. ASAE Paper #90-1054, St. Joseph, MI.

LENTZ, E.M. 1990. Morphological trait effects on digestibility of orchardgrass. Ph.D. Thesis. Iowa State University, Ames. (with D.R. BUXTON).

LORY, J.A. 1990. Quantification of symbiotically fixed nitrogen in the soil surrounding alfalfa roots and nodules. M.S. Thesis, Univ. of MN (with M.P. RUSSELLE).

MATSUSHITA, O., J.B. RUSSELL and D.B. WILSON. 1990. Cloning and sequencing of a *Bacteroides ruminicola* B14 endoglucanase gene. J. Bacteriol. 172:3620-3630.

MCKANE, R.B., D.F. GRIGAL and M.P. RUSSELLE. 1990. Spatiotemporal differences in ^{15}N uptake and the organization of an old-field plant community. Ecology 71:1126-1132.

MEGLIC, V. 1990. Self-incompatibility and Seed Set in Colchicine, Nitrous Oxide and Sexually Derived Tetraploid Red Clover (Trifolium pratense L.). M.S. Thesis. University of Wisconsin, Madison, WI. 36 p. (with R.R. SMITH).

MERTENS, D.R. 1990. Feeding the high producing dairy cow. Proc. Georgia Dairy Herd Mgmt. Conf., p. 43-50.

MERTENS, D.R. 1990. Modeling the relationships among rates of digestion and passage and gut capacity. Proc. Digestive Strategies of Animals. Center for Biol. Res., Nat. Zool. Park, Smithsonian Inst., Washington, D.C. p. 7-9.

MERTENS, D.R. 1990. Using NDF as a measure of forage quality in dairy ration balancing. Proc. Georgia Dairy Herd Mgmt. Conf., p. 6-17.

MEYERS, L.L. and M.P. RUSSELLE. 1990. Spatial and temporal patterns of nutrient uptake in alfalfa and grasses. Agron. Abstr. p. 275.

MUCK, R.E. 1990. Dry matter level effects on alfalfa silage quality. II. fermentation products and starch hydrolysis. Trans. ASAE 33:373-381.

MUCK, R.E. 1990. Predicting lactic acid bacteria on alfalfa. ASAE Paper No. 901523, Am. Soc. Agric. Engineers, St. Joseph, MI.

MUCK, R.E. 1990. Prediction of lactic acid bacterial numbers on lucerne. Grass and Forage Science 45:273-280.

NAGEL, S.A. 1990. Effect of formic acid or formaldehyde treatment of alfalfa silage on nutrient utilization by dairy cows. M.S. Thesis, Univ. Wis. 108 pp. (with G.A. BRODERICK).

NAGEL, S.A. and G.A. BRODERICK. 1990. Rumen flows of microbial and escape protein in cows fed alfalfa silage diets. J. Dairy Sci. 73(Suppl. 1):173 (abstract).

NELSON, W.F. 1990. Impact of stage of maturity and method of preservation of alfalfa on milk production and fiber utilization by lactating dairy cows. Ph.D. Thesis, University of Wisconsin, Madison (with L.D. SATTER).

NELSON, W.F. and L.D. SATTER. 1990. Effect of stage of maturity and method of preservation of alfalfa on production by lactating cows. J. Dairy Sci. 73:1800-1811.

NELSON, W.F. and L.D. SATTER. 1990. Impact of stage of maturity and method of preservation of alfalfa on digestion and passage in lactating dairy cows. J. Dairy Sci. 73 (Suppl. 1):131 (abstract).

NELSON, W.F. and L.D. SATTER. 1990. Milk production in early lactation from first cutting alfalfa cut at two maturities and stored as silage or hay. J. Dairy Sci. 73 (Suppl. 1):131 (abstract).

OLSEN, E.B. 1990. Electrogenic L-malate transport by *Lactobacillus plantarum*: A basis for energy derivation from malolactic fermentation. M.S. Thesis. Cornell University (with J.B. RUSSELL).

OLSEN, E.B., J.B. RUSSELL and T. HENICK-KLING. 1990. Malate transport and lactate efflux by *Lactobacillus plantarum*. Northeast Microbiol. Meeting: Physiology, Ecology, Taxonomy, Troy, NY, June 21-23 (abstract).

PAGE, R.D., W.D. GILSON, L.D. GUTHRIE, D.R. MERTENS and R.C. HATCH. 1990. Serum progesterone and milk production and composition in dairy cows fed two concentrations of nitrate. Vet. Hum. Toxicol. 32:27-31.

PETERSON, T.A. and M.P. RUSSELLE. 1990. The fate of symbiotically-fixed N: A direct assessment for alfalfa. Agron. Abst. p. 277.

PRITZL, P., K.J. SHINNERS and R.G. KOEGEL. 1990. A low energy forage harvester conveying system. ASAE Paper #90-1050, St. Joseph, MI.

QUESENBERRY, K.H. and R.R. SMITH. 1990. Recurrent selection in red clover for plant regeneration from callus tissue. Agron. Abst. p. 202.

RALPH, J. 1990. HyperCard in Chemistry. 199th American Chemical Society National Meeting, 1990 (Boston) Paper CINF-08.

RALPH, J. 1990. Setting up a Bruker Spectrometer for Efficiency and Ease of Use. Bruker NMR Software and Applications Newsletter II(1), 5-20.

RALPH, J. 1990. HyperCard NMR Applications. T.A.M.U. NMR Newsletter 380-5.

ROTZ, C.A. 1990. Emerging technologies in forage harvesting. Proc. 14th Forage Production and Use Symposium. Wisconsin Forage Council. Wisconsin Dells, Wisconsin.

ROTZ, C.A. 1990. Hay conditioning and preservation. Proc Livestock-Dairy-Forage Field Day. Michigan State University, Lake City Experiment Station, Lake City, Michigan.

ROTZ, C.A. 1990. Mechanical and chemical drying aids in humid regions. Proc. 18th National Alfalfa Symposium, p. 4-13. The Certified Alfalfa Seed Council, Inc., Woodland, CA.

ROTZ, C.A. and P. SAVOIE. 1990. Economics of swath manipulation during field curing of alfalfa. Paper No. 90-1049. American Society of Agricultural Engineers, St. Joseph, MI.

ROTZ, C.A., D.R. BUCKMASTER, J.R. BLACK and D.R. MERTENS. 1990. DAFOSYM: an aid for strategic planning of dairy farms. 3rd International Conference on Computers in Agricultural Extension Programs Proceedings. University of Florida, Gainesville, FL.

ROTZ, C.A., R.G. KOEGEL, K.J. SHINNERS and R.J. STRAUB. 1990. Economics of maceration and mat drying of alfalfa on dairy farms. Applied Engineering in Agric. 6(3):248-256.

ROTZ, C.A., R.J. DAVIS and S.M. ABRAMS. 1990. Influence of rain and crop characteristics on alfalfa damage. Paper No. 90-1516. American Society of Agricultural Engineers, St. Joseph, MI.

ROTZ, C.A., R.J. DAVIS, D.R. BUCKMASTER and M.S. ALLEN. 1990. Preservation of alfalfa hay with propionic acid. Paper No. 90-1052. American Society of Agricultural Engineers, St. Joseph, MI.

ROTZ, C.A., R.J. DAVIS, J.W. THOMAS and M.S. ALLEN. 1990. Propionic acid and urea as preservatives for alfalfa hay. 1990 American Forage and Grassland Conference Proceedings. American Forage and Grassland Council, Belleville, PA.

RUSSELL, J.B. 1990. Growth-independent energy dissipation by ruminal bacteria. IN: S. Hoshino, R. Onodera and H. Minato (ed.). The rumen ecosystem: The microbial metabolism and its regulation pp. 23-32, Japan Scientific Society Press-Springer Verlag, Tokyo.

RUSSELL, J.B. 1990. *Lactobacillus plantarum.* Ann. Meeting, Eastern Section Amer. Soc. for Enology and Viticulture, Rochester, NY, July 12-13, 1990 (abstract).

RUSSELL, J.B. 1990. Low affinity, high capacity system of glucose transport in the ruminal bacterium *Streptococcus bovis*: evidence for a mechanism of facilitated diffusion. Appl. Environ. Microbiol. 56:3304-3307.

RUSSELL, J.B. and H.J. STROBEL. 1990. ATPase-dependent energy spilling by the ruminal bacterium. *Streptococcus bovis*. Arch. Microbiol. 153:378-383.

RUSSELL, J.B., H.J. STROBEL and S.A. MARTIN. 1990. Strategies of nutrient transport by ruminal bacteria. J. Dairy Sci. 73:2996-3012.

RUSSELL, J.B., J.D. O'CONNOR, D.G. FOX, P.J. VAN SOEST and C.J. SNIFFEN. 1990. The rumen submodel of the Cornell Net Carbohydrate-Protein System, p. 34-43. Proc. Cornell Nutrition Conference for Feed Manufacturers, Rochester, NY.

RUSSELLE, M.P. and D.L. LINDEN. 1990. Improving recovery of leached nitrate-N by deeply rooted perennial forages. Agron. Abst. p. 46.

SHINNERS, K.J., R.G. KOEGEL and R.J. STRAUB. 1990. Leaf loss and drying rate of alfalfa as affected by conditioning roll type. ASAE Paper #90-1048, St. Joseph, MI.

SMITH, R.R. 1990. Cytological and agronomic characterization of the U.S. NPGS kura clover (*Trifolium ambiguum*) accessions. Proc. XI Trifolium Conference, p. 22, Sublimity, OR. July 9-11, 1990.

SMITH, R.R., F. VERONESI, V. NEGRI and A. GRILLO. 1990. Variation with methods of seed multiplication of introduced trefoil germplasm. Agron. Abst. p. 110.

STROBEL, H.J. 1990. The role of sodium in the bioenergetics of ruminal bacteria. Ph.D. Thesis. Cornell University (with J.B. RUSSELL).

STROBEL, H.J. and J.B. RUSSELL. 1990. Transport and decarboxylation of succinate by a ruminal selenomonad. Ann. Meeting Amer. Soc. for Microbiol., Anaheim, CA, May 13-17, 1990 (abstract).

TAYLOR, N.L., R.R. SMITH and J.A. ANDERSON. 1990. Selection in red clover for resistance to northern anthracnose. Crop Sci. 30:390-393.

TOFTE, J.E. 1990. Reaction of Red Clover (*Trifolium pratense* L. and Diploid Alfalfa (*Medicago sativa* L. and *Medicago falcata* L.) to *Aphanomyces euteiches* DRECHS and Selection for and Inheritance of Resistance in Red Clover. Ph.D. Thesis. University of Wisconsin, Madison, WI, 74 p. (with R.R. SMITH).

UNDERSANDER, D., R.R. SMITH, K. KELLING, J. DOLL, G. WORF, J. WEDBERG, J. PETERS, P. HOFFMAN and RANDY SHAVER. 1990. Red Clover: Establishment, management, and utilization. Wis. Expt. Sta. Bul. A3492. p. 13.

VAN KESSEL, J.S., G. CHEN and J.B. RUSSELL. 1990. Arginine transport by a newly-isolated amino acid fermenting ruminal bacterium. Northeast Microbiol. Meeting: Physiology, Ecology, Taxonomy, Troy, NY, June 21-23 (abstract).

WATTIAUX, M.A. 1990. A mechanism influencing passage of forage particles through the reticulo-rumen: change in specific gravity during hydration and digestion. Ph.D. Thesis, University of Wisconsin, Madison (with L.D. SATTER).

WATTIAUX, M.A., D.R. MERTENS and L.D. SATTER. 1990. Influence of digestion kinetics on increase in specific gravity of forages. J. Anim. Sci. 68 (Suppl. 1):565.

WEIMER, P.J. 1990. Use of mixed cultures for the production of commercial chemicals. IN: J.G. Zeikus and E.A. Johnson, eds. Mixed Cultures in Biotechnology. McGraw-Hill, New York, p. 205-232.

WEIMER, P.J., J.M. LOPEZ-GUISA and A.D. FRENCH. 1990. Effect of cellulose fine structure on the kinetics of its digestion by mixed rumen microorganisms in vitro. Appl. Environ. Microbiol, 56:2421-2429.

WILKENS, P.W., J.T. RITCHIE and C.A. ROTZ. 1990. ALFALFA: A process oriented legume crop model. Agron. Abstr. p. 75.

YANG, J.H., G.A. BRODERICK, R.G. KOEGEL, D.B. RICKER. 1990. Effect of heat treatment of the estimated ruminal escape of protein in alfalfa hay. J. Dairy Sci. 73(Suppl. 1):173 (abstract).